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Synthesis and Testing of Photosensitizer-Folate Conjugates and Potential Photosensitizers for Photodynamic Therapy

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SYNTHESIS AND TESTING OF PHOTOSENSITIZER-FOLATE CONJUGATES AND POTENTIAL PHOTOSENSITIZERS FOR PHOTODYNAMIC THERAPY

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This Ph.D. is dedicated to Wilhelmina C. Trautmann (mother, dec.).

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LIST OF ABBREVIATIONS

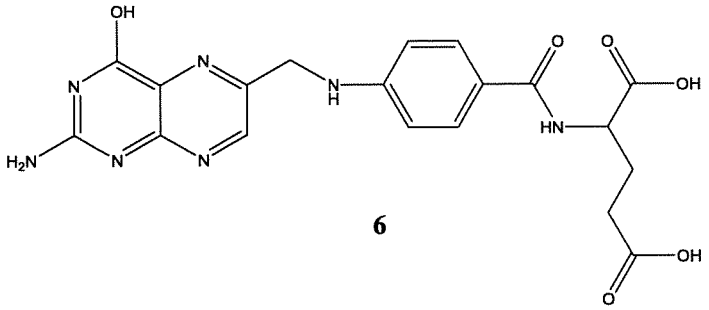
BINAP	2,2'-bis(diphenylphosphino)-1,1'-binaphthyl
CH_2Cl_2	Dichloromethane (methylene chloride)
$CHCl_3$	Trichloromethane (chloroform)
DCC	Dicyclohexylcarbodiimide
DEAD	diethylazodicarboxylate
DMF	*N*,*N*-dimethylformamide
DMSO	Dimethyl sulfoxide
Et_2O	Diethyl ether
HEPES	2-[4-(2-Hydroxyethyl)piperazin-1-yl]ethanesulfonic acid
HOBT	Hydroxybenzotriazole
LAH	Lithium aluminum hydride
LDA	Lithium diisopropylamide
NMR	Nuclear magnetic resonance
PBS	Phosphate-buffered saline
PDT	Photodynamic therapy
PEG	Polyethylene glycol
TBD	1,5,7-Triazabicyclo[4.4.0]dec-5-ene
t-Boc	*tert*-butyloxycarbonyl
THF	Tetrahydrofuran

TLC	Thin layer chromatography
TMS	Tetramethylsilane

ABSTRACT

Photodynamic Therapy (PDT) is a technique that destroys oncogenic (cancerous) cells. Molecules that contain chromophores can function as photosensitizers, and will absorb light at various wavelengths (that being dependent upon the extent of their conjugation). Certain functional groups will form singlet oxygen which is destructive to the cellular environment. Once an in-vitro cell contains a high enough concentration of photosensitizer material and light is able to reach that photosensitizer material, PDT can be used effectively to destroy that cell.

Folate, a metabolite of folic acid **6**, is necessary for DNA biosynthesis, repair, and cell growth. When cells, (cancerous or healthy) are deprived of normal amounts of folic acid and then later introduced to a surplus, their rate of folic acid uptake becomes exaggerated. Under these conditions, cancerous cells, due to exhibiting unregulated proliferation, tend to exhibit folic acid uptake at a significantly greater rate than healthy cells.



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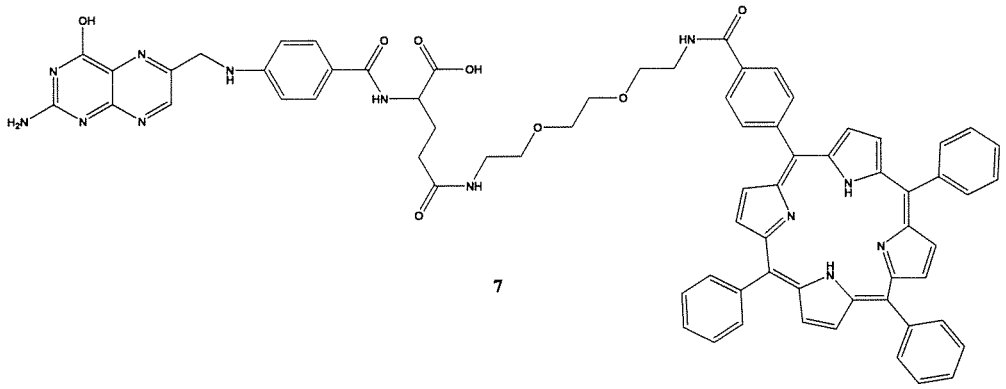
Previously, P. Low *et al.* demonstrated that polyethylene glycol (PEG) can function as a spacer between fluorescent liposomes and folate. Recently, Schneider *et. al.* prepared folate conjugates consisting of small linkers joined to a 4-carboxyphenylporphyrine **7**.



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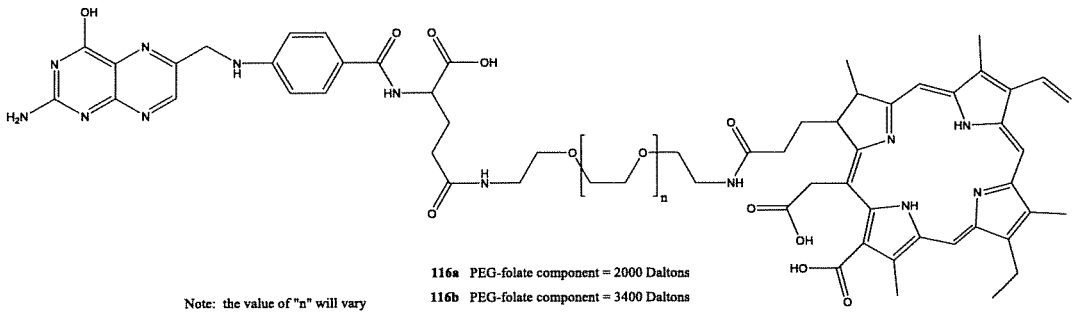
Most recently, L. Donahue reported a PEG-folate conjugate that in-part resembles those prepared by Low, in that it consists of a 2000 ethylenedioxy unit linker, and also in-part resembles those prepared by Schneider, in that it contains a Chlorin-e6 porphyrin photosensitizer. Donahue's target exhibits nearly quantitative death within Hela (immortal-line oncogenic) cells at micromolar concentrations within 120 seconds of exposure to 660 nm light.

This dissertation focuses on preparative synthesis and delivery of folate-linked photosensitizer precursors to folate receptors on cancer cells. Additional Chlorin-e6-PEG-folate conjugates containing ethylenedioxy units approximating 3400, 10000, and 20000 in number are to be prepared (**116b-d**, respectively). Methods of compound analysis are to include proton-NMR, TLC, and melting point. Exposure of these conjugates to near-IR light for 5.0 μM and 0.5 μM concentrations in phosphate buffer saline are to be conducted at intervals of 1 min, 2 min, 4 min, and 6 min.



Note: the value of "n" will vary

116a PEG-folate component = 2000 Daltons

116b PEG-folate component = 3400 Daltons

116c PEG-folate component = 10000 Daltons

116d PEG-folate component = 20000 Daltons

CHAPTER ONE

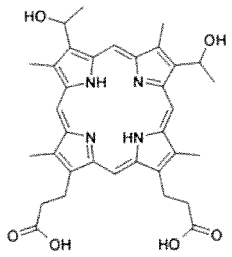
HISTORICAL

Photodynamic Therapy (PDT) is a technique that destroys oncogenic (cancerous) cells. Molecules that contain chromophores can function as photosensitizers, and will absorb light at various wavelengths (that being dependent upon the extent of their conjugation). Certain functional groups, upon absorption of light, can be used to convert O_2 to singlet oxygens which are destructive to the cellular environment. Once an in-vitro cell contains a high enough concentration of photosensitizer material and light is able to reach that photosensitizer material, PDT can be used effectively to destroy that cell.[1-3]

Photodynamic therapy has been employed in several countries since the 1980's, however its origin dates back to 1900. Oskar Raab, a medical student in Munich, Germany, discovered in 1900 that paramecia, when exposed to different dyes in the presence of sunlight, displayed a gradual loss of motion and an inability to divide and replicate. Raab's mentor, Hermann von Tappiener, termed the phenonema "Photodynamische" (meaning photodynamic effect) and proposed that further related studies might one day lead to treatments in medicine.[4-5] Out of this, the technique known today as Photodynamic Therapy emerged.

PDT studies became more widespread in the latter part of the 20th century. In 1975, McDonagh *et al.*, observed cell death when fluorescent bulbs were shined on

glioma cells (brain cells susceptible of becoming cancerous) containing hematoporphyrin **1** (an acid-hydrolyzed product of hemoglobin **2**):



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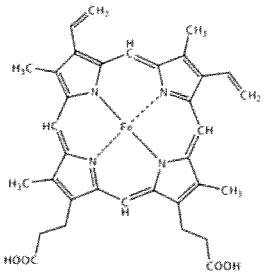

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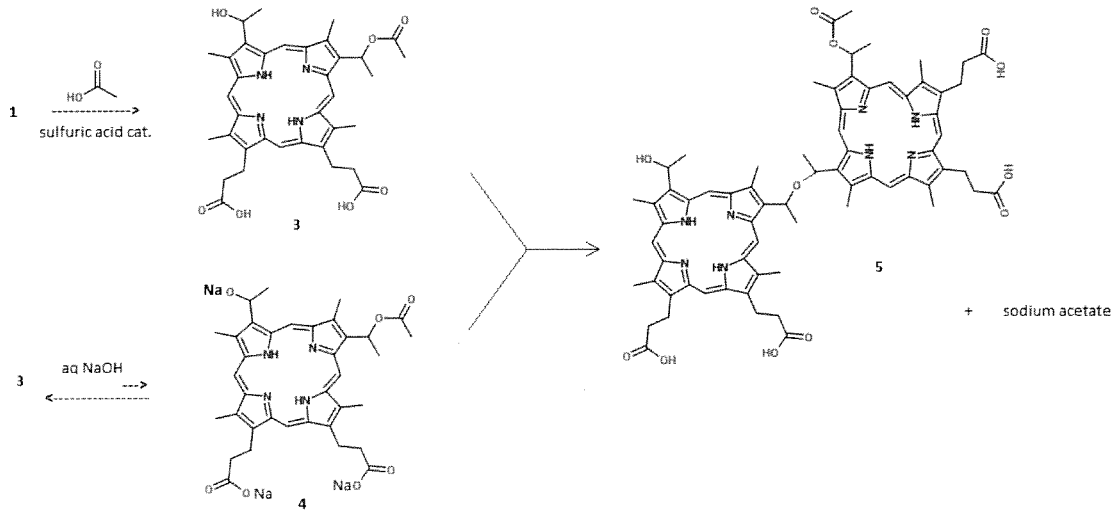
McDonagh extended this methodology to in-vivo studies. Glioma cells containing hematoporphyrin were injected into rats and tumors were allowed to grow from these cells. Upon their subsequent removal, these tumors were destroyed using a 150-watt bulb.[6-7]

About this time, Schwartz was investigating the development of synthetic radiosensitizers (drugs that render tumor cells more sensitive to radiation therapy).[8] The principle behind radiotherapy is that the patient is bombarded with localized radiation in order to promote the formation of DNA-damaging radicals from available oxygen in the cell.[9] Up until this point, radiotherapy had shown limited success, as tumor cells characteristically lack sufficient oxygen to form an abundance of radical oxygen due to their outgrowing of adequate blood supply and becoming partially hypoxic.[10] During his studies, Schwartz discovered that hematoporphyrin tended to localize at sights of neoplasia (tumor growth), where their fluorescence was detectable by UV light. Schwartz later attempted to improve upon these characteristics. He treated hematoporphyrin with a mixture of sulfuric acid and acetic acid, filtered the precipitate,

and subsequently treated the precipitate with aqueous hydroxide to prepare what he termed hematoporphyrin derivative (HPD).[11] Dougherty, who was also interested in radiosensitizers, collaborated with Schwartz, and proposed that HPD was in fact an HP-dimer connected by an ether linkage. The linkage was thought to have been brought about by preliminary formation of an acetate functionality.[12]

Although not directly stated in their publications, it might be suggested that Schwartz and Dougherty had performed a Fischer esterification on one of the two secondary alcohols of an HP monomer to afford **3**. When their material was later treated with hydroxide, deprotonation (to a minor extent in equilibrium, but nonetheless sufficient) of the surviving secondary alcohol on another equivalent of **3** produced **4**. Eventual intermolecular S_N2 substitution between **3** and **4** produced dimer **5**, with water-soluble sodium acetate being the leaving group.



Later, in 1987, a study by Byrne *et al.* revealed through spectroscopic analysis that HPD (now available under the trade name "Photofrin") was comprised of ether linkages, but that the material was mostly HP polymers, with some dimers and trimers mixed in.[13]

Dougherty, a director at the Roswell Park Cancer Center in Buffalo, NY, had discovered during his collaboration with Schwartz that a 400 nm wavelength of light would penetrate tissue only up to approximately 1 cell depth due to high absorption, and that a weaker absorption was required in order for this methodology to be practical from a therapeutic standpoint. Dougherty hypothesized that porphyrins exhibiting absorbances in the 600 nm range might be more appropriate. Patients who had been given HPD via intravenous solution were then exposed to bright red (near IR) light, and a (qualitatively observed) greater reduction in tumor size was observed, supporting Dougherty's hypothesis.[14]

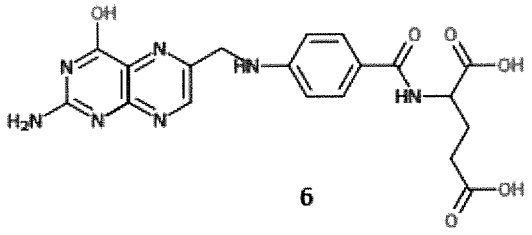
These findings, although pioneering, revealed major limitations of PDT. Three areas in particular that merited further study for the purpose of improving PDT were identified. Scientists began to focus on how to optimize: (1) getting a drug to the target site, (2) getting oxygen to the target site, and (3) getting light to the target sight.[15]

The first area, getting a drug (in this case, a photosensitizer) to the target site, is important because PDT will potentially destroy all cells that uptake the photosensitizer. The near-IR light is intended for tumor cells exclusively; however, even with a narrow beam width, a certain amount of light will reach non-cancerous cells. If the drug reaches neighboring healthy tissues, then healthy cells become susceptible to unintended

eradication. Even if the beam is perfectly targeted, toxicity levels may build up from the drug that lingers in healthy tissues.[16]

Low's research group (Purdue University, IN) focused their efforts on searching for ligands that would bind selectively to cancerous cells. This strategy enabled the exploration of many different types of potential anti-cancer drugs, including protein toxins and liposomal carriers of chemotherapeutics. Low had learned that biotin (a vitamin that breaks down carbohydrates and fats in the blood), when attached to proteins, was capable of being brought into cells by endocytosis. He began investigating the use of vitamins as an alternate delivery method, along with other compounds of biological significance. These compounds exhibited varying success of delivery, but what consistently lacked with the delivery of these compounds was selectivity for cancerous cells.[17]

Low became aware that folic acid (vitamin B_9) (**6**) is an important material for cellular function. In particular, folate, a metabolite of folic acid, is necessary for DNA biosynthesis and repair, and ultimately for cell growth. When cells, (cancerous or healthy) are deprived of normal amounts of folic acid and then later introduced to a surplus, their rate of folic acid uptake becomes exaggerated. Under these conditions, cancerous cells, due to exhibiting unregulated proliferation, tend to exhibit folic acid uptake at a significantly greater rate than healthy cells.[18]



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Low's study shifted towards preparative synthesis and delivery of folate-linked photoreceptors to folate receptors on cancer cells. Folic acid was selected as a targeting ligand largely due to its high affinity for the folate receptor (even after the potential photosensitizers selected by Low were linked to it) and also due to the fact that there is a much greater abundance of folate receptors on cancerous cells than there are on normal cells.[19] This latter fact (significant uptake by cancer cells) became prominent in the early 1990's, when studies independent of one another by Weitman *et al.*, Coney *et al.*, and Campbell *et al.*, revealed that several monoclonal antibodies that were used to identify cancer cells in tissue biopsies also recognized folate receptors; hence, the conclusion was drawn that the folate receptor was predominantly found in oncogenic tissue.[20]

Taking what he had learned from his previous research, Low decided to link proteins to folate. When momordin (melon protein) was linked to folate, 50% efficacy for inhibition of function (IC_{50}) within cancer cells was observed at a dosage of 10^{-9} M. Successful use of pseudomonas exotoxin (a ribosome-inactivating protein) was found to be dependent upon the specific organic functional group selected to link the folate and the protein together. When a reducible disulfide bond was used, an IC_{50} of 10^{-11} M was observed, but when a thioether was used, a decline by over 4 orders of magnitude was

observed. Thus, having a linker that was readily cleavable was to be included in Low's future research.[21]

Low began using polyethylene glycol (PEG) as a spacer between fluorescent liposomes (spherical vessels capable of serving as a vehicle for nutrients and pharmaceuticals) and folate, as he had found that liposomes linked directly to folate were inefficient at binding with folate receptors. Furthermore, the reticuloendothelial system, which serves as a dead-cell and toxic particle scavenger, efficiently collects those conjugates which lack a PEG unit, thus, not giving them adequate time to circulate in-vivo. Conversely, over 200,000 PEG-liposome-Folate conjugates were reported to have entered cancer cells. In short, Low found that better tumor-specific delivery could be obtained by using short PEG chains (less than 2000 units in length) with small-size liposomes (less than 100 nm in diameter).[22] Low, in collaboration with scientists at Endocyte, also explored the use of short-chain peptide spacers (as an alternative to PEGs) in an effort to further increase water solubility of potential drugs containing folate and disulfide functionalities. One of their targets contains a Glu-Asp-Arg-Asp-Asp-Cys sequence, and is in clinical trials.[23]

Low then drew importance to the discovery that folate-tethered proteins are not digested following internalization, and his studies included analysis of their cytotoxicity. Imaging studies, particularly multiphoton intravital microscopy (a technique permitting visualization of dynamic biological events within tissues of anesthetized living animals) with folate-Texas Red as a model, revealed that folate-conjugate build-up was observed in the kidneys due to binding with the apical (outer or top) surface of proximal tubule

cells; but that subsequent transcytosis (vesicle transport of macromolecules within the interior of a cell) of the conjugate across the basal membrane (bottom tissue surface) led to release of vesicle contents into the blood stream, thus, returning the conjugates to the circulatory system within minutes. Kidney toxicity has not been observed with any folate conjugates tested to-date; and Low suggests that should future conjugates exhibit kidney toxicity, predosing with antifolates would significantly reduce kidney uptake of folate conjugates without significantly reducing oncogenic selectivity and uptake.[24]

The second area of PDT research meriting further study, getting oxygen to the target site, has been of interest since Warburg's research earned him the Nobel Prize in 1931 for demonstrating that an inverse relationship exists between the abundance of oxygen within cells and their tendency to become oncogenic, with a 60% or greater reduction in oxygen abundance being a threshold for when cancer cells can be expected to emerge.[25] Normal cells meet their energy needs by respiration of oxygen; but when toxins build up around the cellular environment, damage to respiratory enzymes occurs, and cells are rendered incapable of performing their designated functions. Their roles become only to stay alive, and without the ability to perform respiration of oxygen, they rely on fermentation of sugar as their primary source of energy. Sugar fermentation produces lactic acid, which, upon build-up, becomes toxic, and interferes with oxygen transfer, thus, further promoting sugar fermentation as part of a cycle.[26]

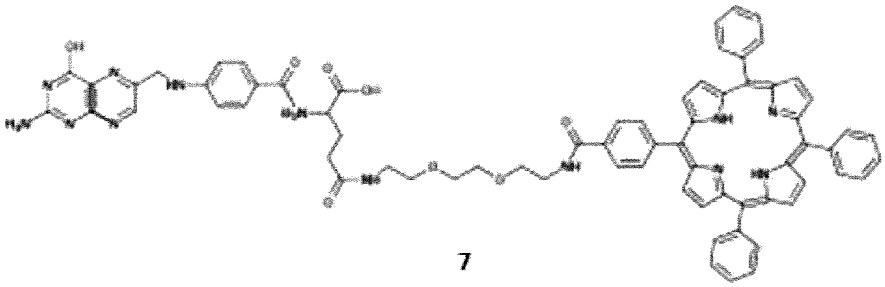
Today, many products designed to deliver supplemental oxygen to the target site are available, but fall short of society's expectations as cancer remedies. Some of these products do manage to increase the oxygen levels in the blood, and thus, eventually

increase oxygen levels surrounding the cells; but they are incapable of promoting the active transport of that supplemental oxygen into the cells themselves. To circumvent this limitation, a product known as Oxygen Elements Plus advertises that it initiates reactions that take place inside of the cell, thus, producing intracellular oxygen. Hydrogen is mentioned as being a by-product. The proposed reaction and the proposed mechanism of action behind Oxygen Elements Plus is under patent and not available to the public domain; but the manufacturers do advertise that Oxygen Elements Plus consists of a mixture of hydrogen sulfate, acids, trace minerals, amino acids, and enzymes.[27]

Yang *et al.* at the MOE Key Laboratory of Laser Life Science & Institute of Laser Life Science has looked into both getting oxygen to target areas and also getting light to the target site. In his studies, Yang discovered that during PDT, vascular closure reduces oxygen supply to targeted tissues. Patients with inadequate blood circulation or who have excessive clumping of red blood cells have a slower bloodstream flow and that in turn slows oxygen delivery. In an effort to combat this, he administered heparin (an anticoagulant) prior to exposing tissues to irradiation, with Photofrin as the photosensitizer. Yang observed a significant reduction in thrombosis (blood clotting) during light irradiation, with an improvement in blood perfusion, oxygen supply, and light delivery to targeted tissues, and attributed these findings to an increased production of local reactive oxygen species.[28]

Within the last decade, Schneider *et al.* has prepared folate conjugates consisting of small linkers joined to a 4-carboxyphenylporphyrin. Variations in linkers include a

hexa-1,6-diamine and a 2,2'-(ethylenedioxy)-bis-ethylamine (the latter conjugate, **7**, shown below):



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In his studies, Schneider discovered that a significant difference in photodynamic activity was observed when using the hexa-1,6-diamine linker versus the ethylenedioxy linker (the molar extinction coefficients in ethanol at 650 nm were 1266 $M^{-1}cm^{-1}$ and 2373 $M^{-1}cm^{-1}$, respectively); however, the two targets afforded nearly identical fluorescence quantum yields (9.8% and 9.9%, respectively). Schneider summarizes that folate selectivity is linker-independent, with both targets showing a 7-fold greater accumulation in cancer cells over that of pure tetraphenylporphyrin (24 hour incubation using KB cells overexpressing the folate receptor).[29]

Most recently, L. Donahue at Loyola University Chicago has reported a PEG-folate conjugate that in-part resembles those prepared by Low, in that it consists of a 2000 ethylenedioxy unit linker, and also in-part resembles those prepared by Schneider, in that it contains a Chlorin-e6 porphyrin photosensitizer. Donahue's target exhibits nearly quantitative death within HeLa (immortal-line oncogenic) cells at micromolar concentrations within 120 seconds of exposure to 660 nm light. Pure Chlorin-e6 was also shown by Donahue to kill cells (although to a lesser extent than the conjugate); however,

its mechanism of action is thought to involve binding on the exterior of the cell membrane, as folate receptors do not recognize free-chlorin and thus, do not subsequently invaginate it by endocytosis.[30] As to whether the binding of free-chlorin promotes cell death via competitive or non-competitive inhibition is a topic of future study.

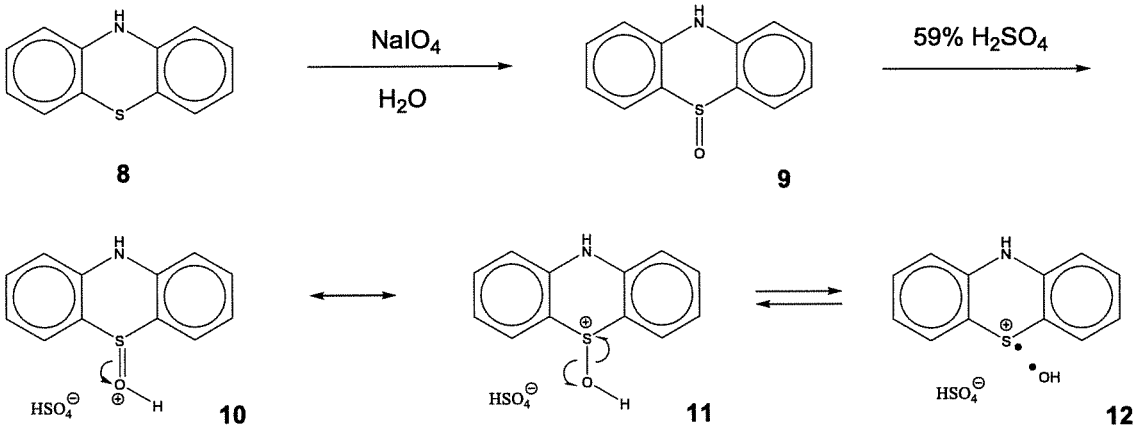
CHAPTER TWO

METHODOLOGY AND RESULTS

Dyes, as well as porphyrins, have been studied[31] for use as photosensitizers in PDT. Porphyrins contain macrocyclic systems that coordinate with various metal cations through heteroatom ligand formation. Dyes are not inherently macrocyclic; and they tend to have a lower molecular mass, making them more applicable for oral bio-availability.

Phenothiazine **8**, a relatively small chromophore-containing dye, can be amide-linked to folic acid with the appropriate carbon tether to create a molecule that has a molecular mass less than 700 g/mol. Additionally, the nitrogen and the sulfur functionalities of phenothiazine offer opportunity for N-O and/or S-O oxide formation, enabling photo-induced generation of destructive oxygen radicals.

Laboratory preparation of destructive radicals (*e.g.* **12**) for the purpose of *in vitro* studies has been reported.[32]

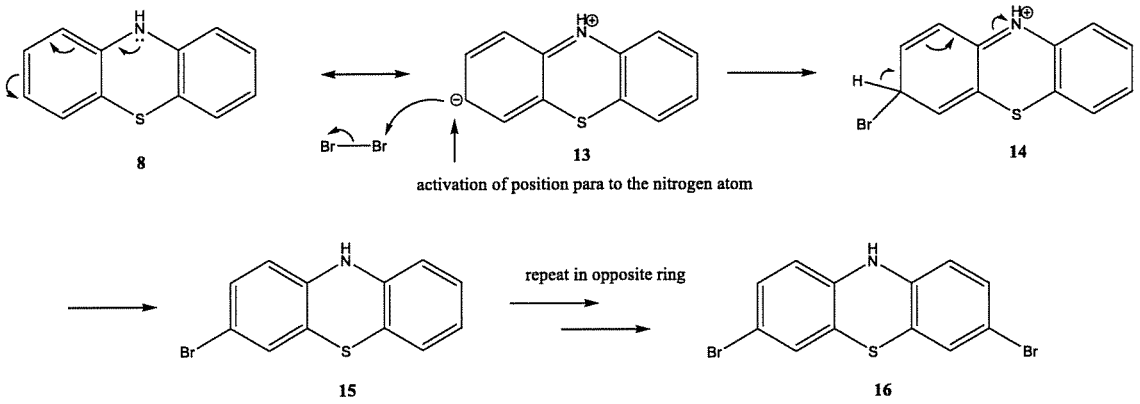


Sodium periodate is known to selectively oxidize aliphatic and aromatic sulfides to sulfoxides in the presence of amines, thereby converting **8** into **9**.[33] The iodine atom that sodium periodate contains is hypervalent with a +7 oxidation level, enabling it to accept electrons and undergo reduction during the sulfide oxidation process. Protonation of **9** with sulfuric acid creates conjugate acid **10**, which contains a resonance stabilized oxonium cation octet capable of giving rise to resonance hybrid contributor **11**. Homolytic cleavage of **11** generates sulfur radical-cation heptet **12**, paired with its hydroxyl radical that is destructive to cells.

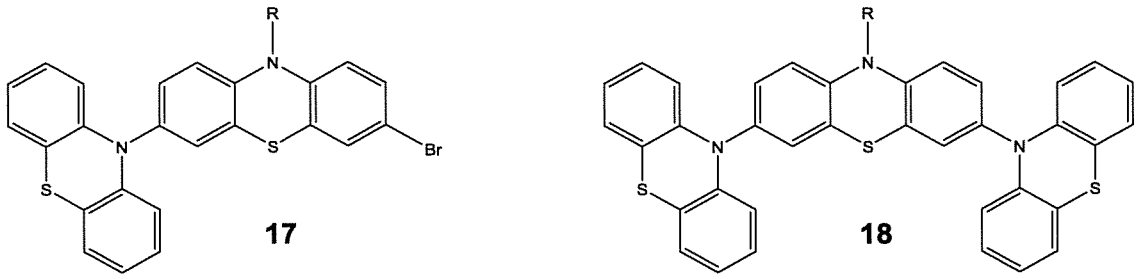
The cells of skin-visible tumors are close to the skin surface, enabling skin-penetrating light to reach inside the cells. By introducing phenothiazine-tethered folamides to the cells of a tumor, it was envisioned that PDT would be able to effectively destroy tumor cells.[34-35]

Earlier studies by Sanjeevaiah have shown that **8** shows UV activity, but not sufficient to exceed hemoglobin's maximum absorbance of 421 nm.[36] The objective was to synthesize a target that absorbed beyond 600 nm to ensure that adequate absorption by the sensitizer was possible. It has been reported that halogenation of aromatic systems can generate bathochromic (increasing wavelength) shifts observable in the UV spectrum.[37] Both bromine and chlorine are known to substitute on aryl systems; however, bromine is a liquid at room temperature and thus, easier to use in comparison with gaseous chlorine. Use of liquid bromine in carbon tetrachloride at room temperature converted phenothiazine to 3,7-dibromophenothiazine **16**. The regioselectivity of this

dibromination is attributed to the ortho-para directing properties of the conjugated electron donating nitrogen atom.

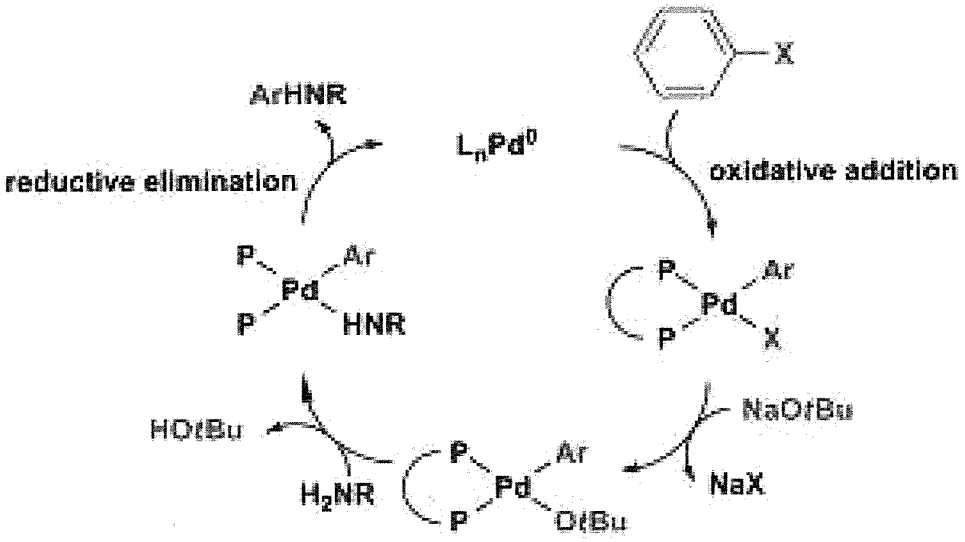


UV analysis of **8** versus **16** in methanol revealed that maximum absorbance had shifted from 252 nm to 326 nm, respectively. This does indicate a bathochromic shift; however it is not of sufficient magnitude for our photosensitizers to absorb beyond hemoglobin. The new availability of halide leaving groups at these positions, however, created the option to prepare phenothiazine dimers **17** or trimers **18** with longer wavelengths.



It was envisioned that synthesis of trimer **18** (with R = H) could be accomplished via Buchwald-Hartwig and/or Ullman coupling methodology. The Buchwald-Hartwig transformation involves a coupling between aryl halides and primary or secondary amines.[38] Nucleophilic aromatic substitution (S_NAr) reactions have limited success due to both the nucleophile and the electrophile having electron-rich character, and thus,

repelling one another. The strategy behind Buchwald-Hartwig conditions is, in a sense, to gradually "work" the nitrogen-based nucleophile onto the aryl system in a stepwise fashion. A single halide is generally not sufficiently electron withdrawing for ipso amine attack to occur on a pi-electron-rich aryl system, so prior dissociation of the leaving group from the aryl system is employed by oxidative addition.[39]



The initial step in the proposed mechanism for the Buchwald-Hartwig process is referred to as an oxidative addition because palladium metal is transitioning from an oxidation state of zero to an oxidation state of +2. It occurs by palladium insertion into the carbon-halogen bond of the aryl halide, which reduces the oxidation state of both the carbon atom and the halide atom. Halides (other than fluorine), being conjugate bases of classical strong acids, are weakly basic, and thus, potential leaving groups. With a transition metal now being the group directly bonded to the ring, the chemistry behind replacing the leaving group is altered.

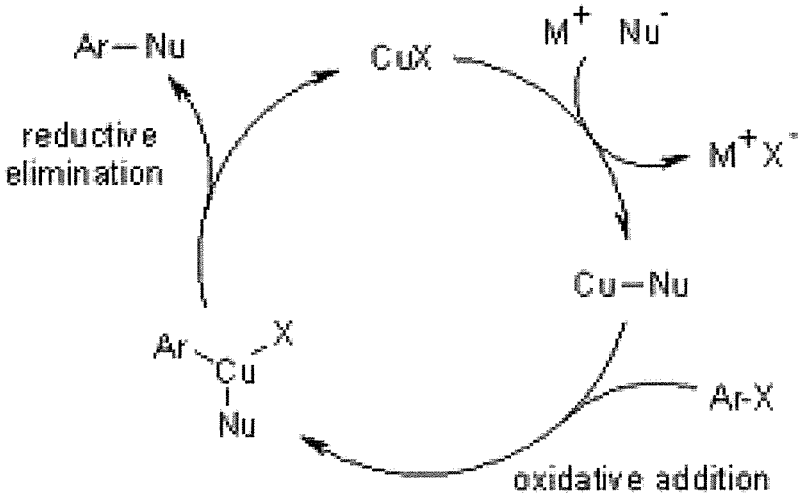
Direct nucleophilic attack by an electron-rich atom is no longer required at the electron-rich ipso position. Instead, the transition metal can be removed by reductive elimination, a process opposite to how it was initially introduced onto the ring. Sodium tert-butoxide offers an oxygen anion which is nucleophilic enough to displace the halide on the palladium, while simultaneously offering sufficient steric hindrance to minimize a second equivalent of alkoxide from bonding with palladium. Precipitation of a xylene-insoluble sodium halide salt by-product drives completion of this conversion by Le Châtelier's principle.

Nitrogen, being less electronegative than oxygen, has a more polarizable electron cloud, and the desired amine nucleophile can now favorably exchange with the alkoxide. *Tert*-butyl alcohol forms as an immiscible layer, which dissolves unreacted sodium *tert*-butoxide and minimally dissolves prior-formed halide salts.

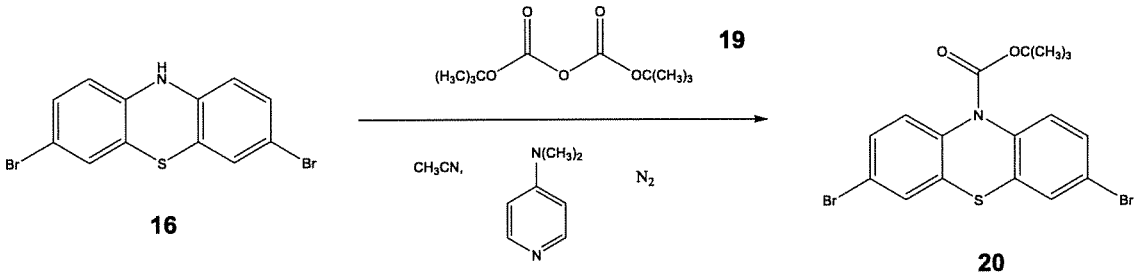
The final step of the process, reductive elimination of the palladium atom, restores the palladium reagent to its original oxidation state of zero while coupling the nitrogen to an aryl system that is less electron rich than it was before the carbon-nitrogen bond formed. The fact that the palladium reagent is never consumed but continually recycled during the course of the reaction enables it to be used in catalytic amounts. Furthermore, post-reaction recovery of the palladium reagent via filtration and rinsing enables its re-use, introducing cost-efficiency as part of the Buchwald-Hartwig methodology.[40]

Classical Buchwald-Hartwig conditions require elevated temperature in order to increase reagent solubility. The system can be put under pressure in order to favor the initial addition pathway.

The Ullmann reaction has also been used in successful couplings between aryl amines and electron-rich aryl iodides. The Ullmann had been thought to proceed via nucleophilic aromatic substitution between an aryl halide and an *in situ* prepared organocopper reagent, but the formation of Cu(I) salts suggested that a mechanism similar to the Buchwald Hartwig reaction was occurring.[41]

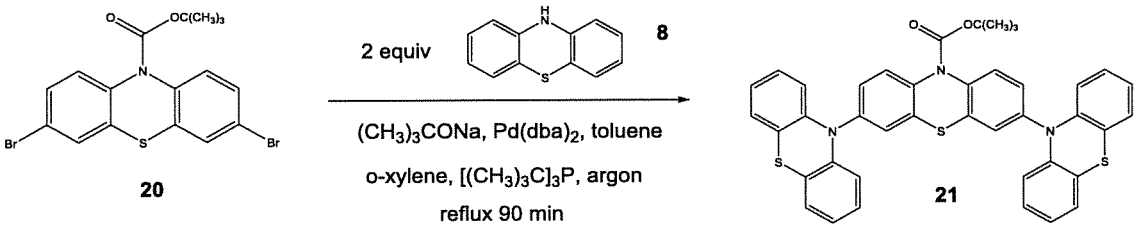


In order to avoid an already brominated phenothiazine from acting as a nucleophile during the Buchwald-Hartwig or Ullmann reaction, we invoked carbamate protecting group strategy.

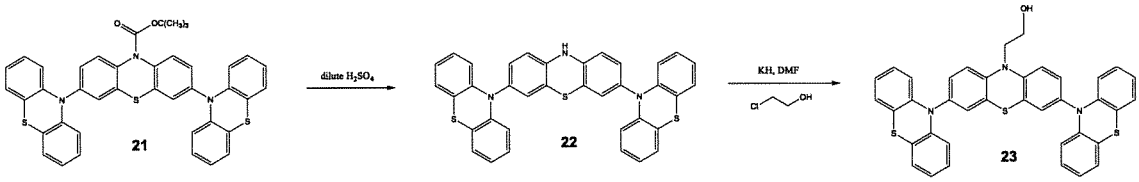


4-(*N*,*N*-dimethylamino)pyridine was introduced as a base to deprotonate the nitrogen atom of **16**, and subsequent carbonyl attack of di-*tert*-butyl dicarbonate **19** led to the formation of t-boc-protected 3,7-dibromophenothiazine **20**.

Treatment of **20** with two or more equivalents of phenothiazine in the presence of tri-*tert*-butyl phosphine, sodium *tert*-butoxide, palladium(0) bis(dibenzylideneacetone) (a ligand to facilitate cross-coupling reactions), and *o*-xylene/toluene under argon at reflux under pressure enabled Buchwald-Hartwig coupling at positions 3 and 7 to form a boc-protected phenothiazine trimer **21.**

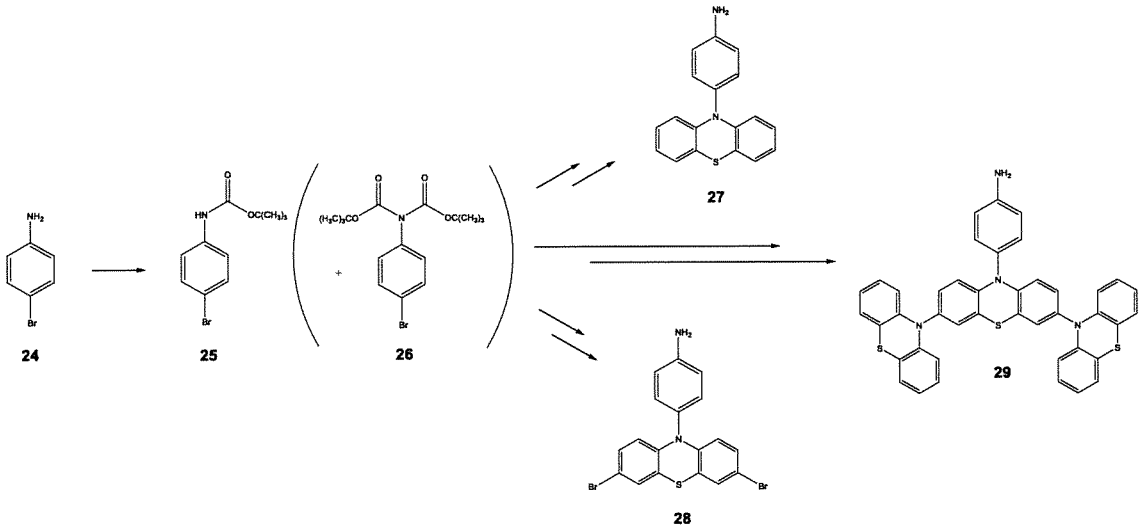


The boc-protecting group of **21** was later removed by Sanjeevaiah at Loyola University Chicago under acidic conditions to produce free-amine trimer **22**, which he subsequently linked with 2-chloroethanol to create a short ethylene-tethered adduct **23**.



This tether, however, proved to be inadequate in length for final esterification with folate's gamma carboxylic acid functionality.[42]

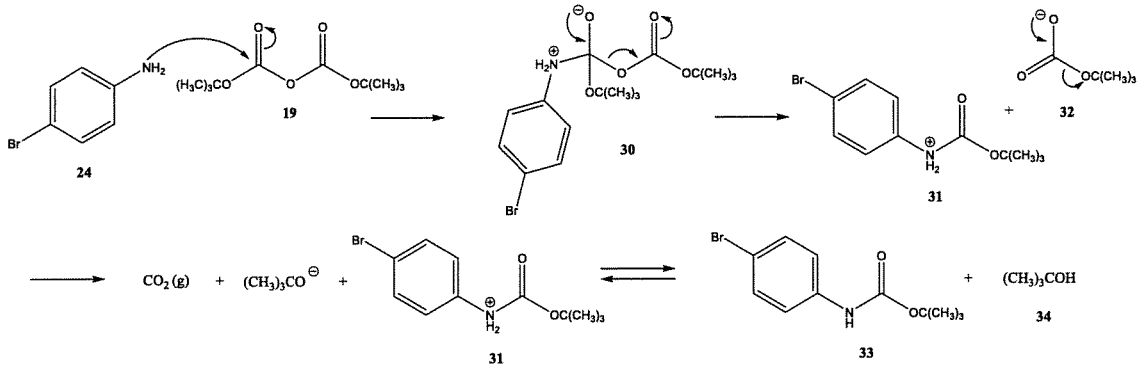
4-Bromoaniline **24** was then proposed as a suitable tether, given its size and planarity. Boc-protection of **24**, subsequent cross-coupling with **8**, **16**, or **22**, and finally, deprotection, was planned, in order to prepare **27-29**.



This approach, when attempted, revealed differences between how **8** (and **16**) behaved during this multi-step synthetic methodology, in comparison with how **24** behaved.

Phenothiazine (**8**) is a secondary aryl amine. Upon boc-protection, its nitrogen atom becomes tertiary. This nitrogen atom's non-bonding electron pair is now not only delocalized with the aromatic pi-electron system of the sensitizer, but also with the carbonyl pi-electron system of the carbamate. This diminishes the availability of nitrogen's non-bonding pair to function as a nucleophile and to become di-protected.

4-Bromoaniline **24**, in comparison, is a primary amine. It is capable of participating in both a first and a second N-acylation without formation of an ammonium cation. Di-*tert*-butyl dicarbonate **19**, upon reacting with amine nucleophiles, is converted into *tert*-butylcarbonate **32**, an intermediate which decarboxylates into carbon dioxide gas and water-soluble *tert*-butoxide.

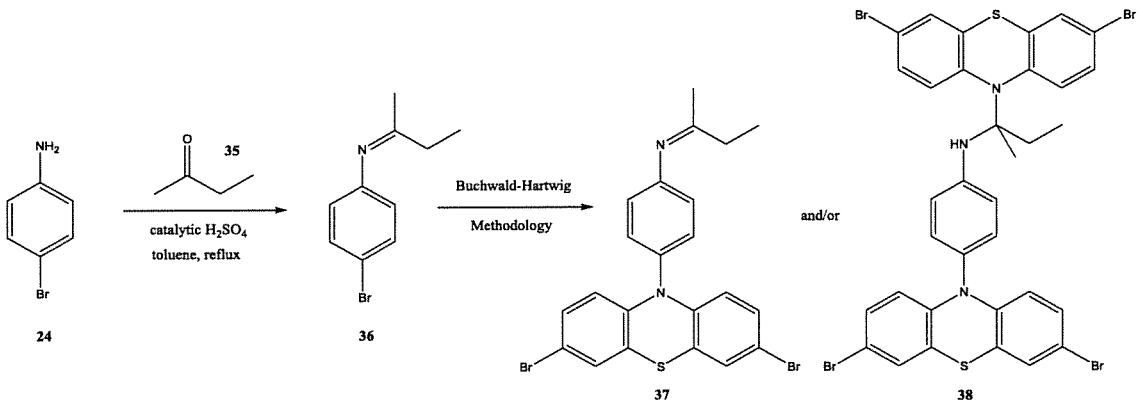


Given that extraction can remove water-soluble impurities, use of reagent **19** in excess molar equivalents with respect to **24** seemed preferred, with the intention of preparing **26** exclusively. This approach, however, resulted in a mixture of mono- and di-protected 4-bromoanilines (**25** and **26**, respectively). Reducing the number of equivalents of **19** to a ratio closer to that of 1:1 stoichiometry with respect to **24** did not provide **25** exclusively, but instead resulted in a mixture of **25** with unreacted **24**. Given the sensitivity of Buchwald-Hartwig couplings to impurities, purification of **26** became necessary.

Carbamates are generally stable to base, but labile to acid. Silica gel is acidic, rendering carbamates susceptible to deprotection during chromatography.[43] Attempts had been made by Sanjeevaiah to purify previously prepared carbamates (1) through the use of basic pH eluent (or silica pre-soaked with ammonium hydroxide), and (2) via recrystallization, but proved to be only marginally successful. Given that a purification technique that would adequately separate **26** from **25** and/or from unreacted **24** had not been developed, attempts to prepare **27-29** were postponed.

Imine synthesis was next considered as an alternative to carbamate protection, as its deprotection could readily be brought about by hydrolysis. Furthermore, imines serve as electrophiles for a variety of nucleophiles, some of which could be used to link

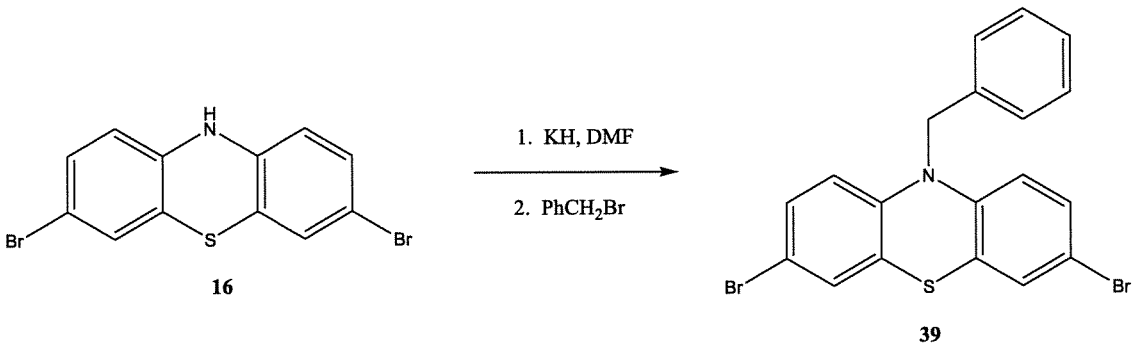
additional sensitizer-containing substituents to our compound. The sodium *tert*-butoxide base used during Buchwald-Hartwig coupling would presumably be too sterically hindered to nucleophilically attack imines prepared from ketones with tetrahedral side chains. Roaming phenothiazine might be able to attack the imine functionality, but aminal formation would occur rather than deprotection, and in the process a molecule with potentially greater UV wavelength, **38**, would be formed.



Preparation of **36** was attempted by reacting **24** with 2-butanone at reflux. A Dean-Stark trap was filled with toluene and added for the purpose of collecting water by-product as it formed. The equilibrium between ketone starting material and imine product would then be shifted toward replenishing the deficient water by-product, and in the process, convert all unreacted ketone to imine. Water volume could be measured, and prior calculation of water's theoretical yield would enable the experimentalist to determine when quantitative conversion had been reached, and thus, when to discontinue heating. The collection of water by-product was not observed, however, and proton NMR revealed that unreacted starting material was present. The transformation was repeated with the addition of catalytic concentrated sulfuric acid, however no change was observed. We attribute these

results to nitrogen's conjugation with the aromatic ring diminishing both its nucleophilicity towards the carbonyl and also its ability to displace the water leaving group.

Benzyl protection was another available option, but had been postponed until this stage because deprotection of benzyl amines is known to be less facile in comparison with other protecting groups. Dibromophenothiazine **16** was deprotonated with potassium hydride, followed by addition of benzyl bromide, to make **39**.



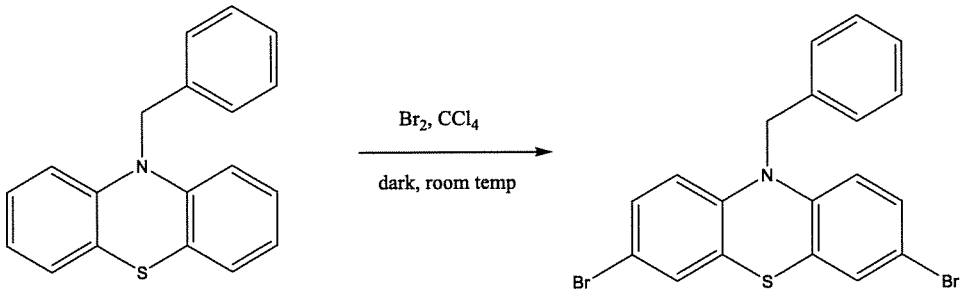
16 **39**

The available potassium hydride was in the form of a slurry-suspension: 30% KH/mineral spirits. Pre-lab calculation of molar equivalents was based on this ratio. Mineral spirits are high molecular weight hydrocarbons (e.g., hentriacontane) that prevent humidity from protonating hydride anion to produce hydrogen gas and potassium hydroxide, a weaker Bronsted-Lowry base. Hexane or petroleum ether (a mixture of low molecular weight alkanes generally less expensive than hexane) should be used (immediately prior to conducting the reaction) to rinse away the mineral spirits in order to prevent unwanted dilution and diminished collision frequency. *N*,*N*-dimethylformamide (DMF), a polar aprotic solvent, is highly miscible with water, and should be freshly distilled from calcium hydride to minimize water content. Unused post-distilled portions of DMF

should be stored over oven-dried molecular sieves. Note: Even when freshly distilled, DMF tends to contain trace amounts of water and it will fizzle (indicating hydrogen gas production) upon coming into contact with hydride anion.

In an effort to develop a methodology suitable for incorporation into the undergraduate laboratory curriculum, the non-pyrophoric potassium tert-butoxide was used in place of potassium hydride. These alternate conditions, however, produced mixtures of mono and dibenzyl-protected 4-bromoanilines. This discrepancy is attributed to potassium *tert*-butoxide's significantly lower basicity, in comparison with potassium hydride (pK_a of conjugate acids: *tert*-butyl alcohol = 18, hydrogen gas = 35).[44] Increasing reaction duration with potassium *tert*-butoxide might lead to dibenzyl-protection with yields similar to those obtained with potassium hydride, however this would detract from its utility as an undergraduate experiment. Undergraduate laboratory periods are generally limited to four or less hours, and equipment must be shared between sections, preventing experiments from being conducted overnight. As a safety precaution, benzyl bromide should be handled in the hood to avoid difficulties with respiration; and splash goggles, (rather than safety glasses) should be worn to provide adequate protection against this severe lachrymator.

As an alternative route to **39**, it was shown that phenothiazine **8** undergoes benzyl protection with benzyl bromide to make **40**, and subsequent dibromination will successfully prepare the desired **39**.



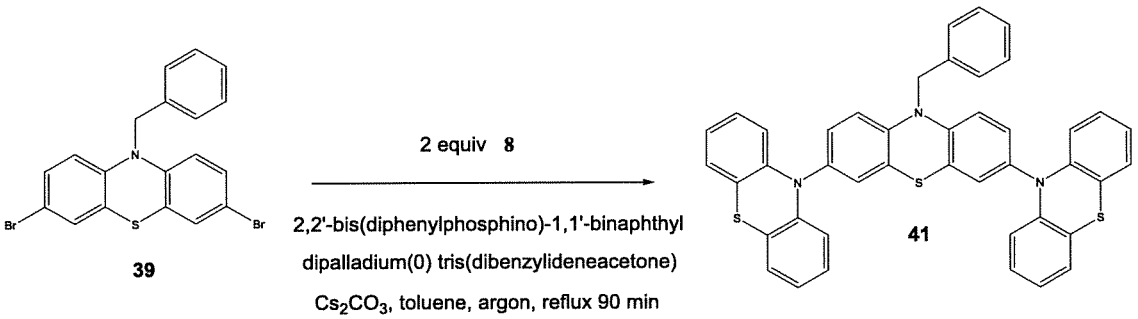
40 **39**

The bromination requires sodium bicarbonate as a proton scavenger (to prevent any HBr generated *in situ* from protonating the phenothiazine nitrogen atom, making it an amminium ion and subsequently changing the regioselectivity of the electrophilic aromatic substitution) and also requires the absence of light to prevent unwanted bromine radical formation.

The option to prepare **16** versus **40** enroute to **39** is at the discretion of the experimentalist; however, Thin Layer Chromatography (TLC) revealed that numerous relatively polar impurities (some of which were not unreacted starting reagents) were present when bromination was employed as the initial step in the two-step synthesis. Note: Use of benzyl chloride in place of benzyl bromide was also demonstrated; however the reaction duration was slightly increased to ensure complete conversion. Chlorine exhibits greater electronegativity in comparison with bromine, making chlorine a more compact atom, with better size matching and orbital overlap to carbon. The carbon-chlorine bond is stronger than the carbon-bromine bond, and chlorine is a poorer (and thus, less kinetic) leaving group relative to bromine.

Preparation of the new phenothiazine trimer was the next step towards reaching a target. Buchwald-Hartwig conditions, however, were found not to be standard from the

previous system to this system. Conditions used for preparing **21** from **20** proved unsuccessful in preparing **41** from **39**.



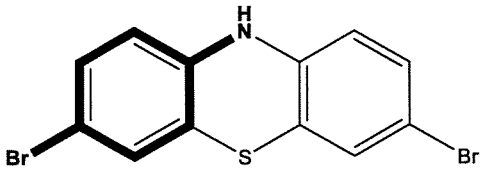
It has been reported that ligands, bases, and solvents must be altered in order to accomplish Buchwald-Hartwig couplings.[45] Varying amounts of cesium carbonate, ligand 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (BINAP), dipalladium(0) tris(dibenzylideneacetone), and phenothiazine in varying concentrations of toluene were used in repeated attempts before product synthesis was achieved. It is noteworthy that Buchwald-Hartwig reactions, even when heated, proceed as suspensions, not as solutions. Phenothiazine exhibits adequate solubility in toluene, however $Pd_2(dBa)_3$ and BINAP do not. Trimer **41**, when formed, requires chromatography (gradient eluent beginning at 5% diethyl ether/petroleum ether, increasing in polarity), with as many as eight compounds appearing when the crude product mixture is analyzed by TLC.

For comparison with Buchwald-Hartwig methodology, and in an effort to prepare trimer **41** with fewer impurities, Ullmann coupling conditions were employed. Cesium carbonate, cuprous iodide, and acetonitrile were refluxed with excess phenothiazine. Filtration was used to separate the water-insoluble cuprous iodide from the rest of the suspension. No apparent product formation occurred. Sodium iodide with solid copper

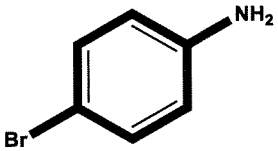
beads was then used in place of cuprous iodide, and potassium phosphate was added as a buffer. Again, product formation was not observed. Copper(II) acetate was then used in place of the solid copper beads without success. Eventually, it was determined by A. Romich at Loyola University Chicago that the use of Copper(I) catalysts were necessary in order to generate the desired product.

Traditionally, Ullmann work-ups employ brine washes in order to prevent product from transferring into the aqueous layer in trace amounts.[46] Introducing **41** to brine, however, led to the precipitation of cesium carbonate salts and the appearance of emulsions. Consequently, brine washes were omitted in subsequent Ullmann experiments.

Even following purification, **41** proved challenging to characterize by proton NMR analysis. Comparison of **16** with 4-bromoaniline **24** revealed that both structures have a nitrogen atom with a pair of non-bonding electrons that can function as an electron donor to a benzene ring, and a bromine at the position para to the nitrogen [Note: The bold-type is for the purpose of emphasizing structural similarities. It is not meant to depict wedge-dash stereochemistry.]



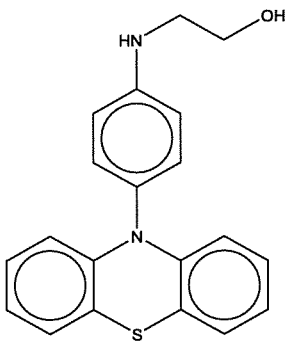
16



24

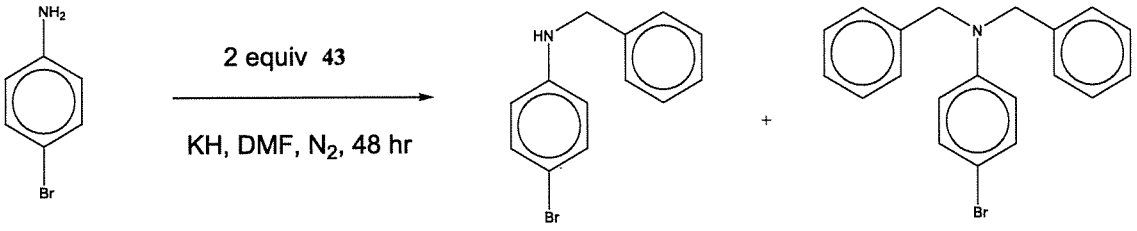
Based on this pattern, it was envisioned that 4-bromoaniline could be used as a multi-faceted model system for (1) optimizing conditions to bring about the protection of

primary amines, (2) optimizing conditions to bring about Buchwald-Hartwig couplings, (3) simplifying the aromatic region of proton NMR spectra for the purpose of later recognizing these same identifiable patterns in more cluttered aryl regions of Buchwald-Hartwing products, particularly trimers, and (4) creating a two-dimensional spacer between phenothiazine sensitizers (**16** and **18**) and the original ethanolamine tether **42** proposed by Sanjeevaiah that was intended for esterification with folate.



42

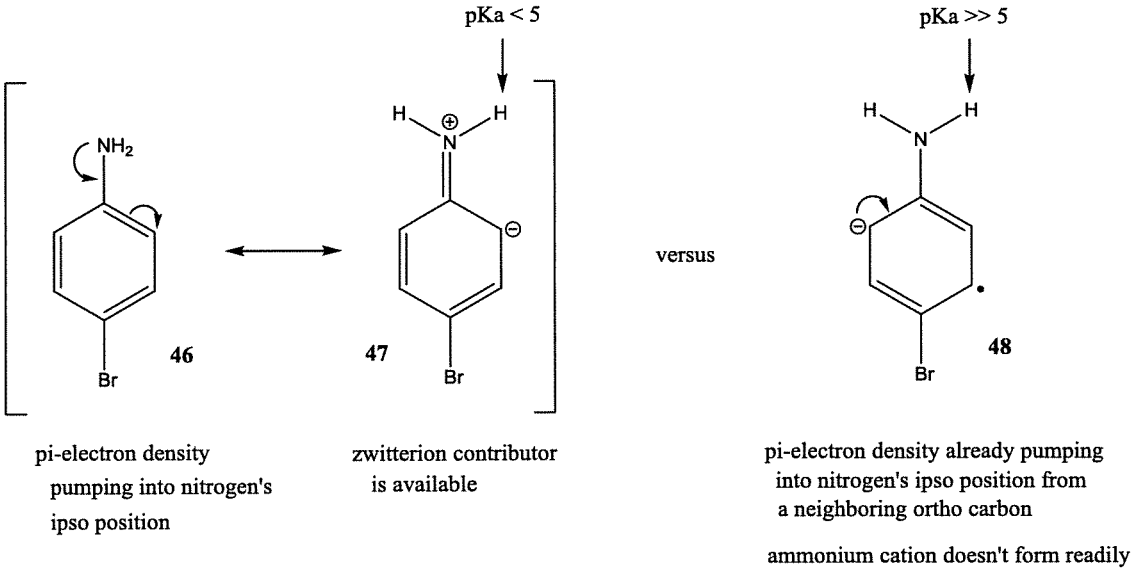
Analogous to the protection of the phenothiazine systems, benzyl bromide (**43**) was employed to protect 4-bromoaniline, with different equivalents of benzyl bromide being used to make the mono-protected **44** and di-protected **45** adducts.



24 **44** **45**

It is noteworthy that the potassium hydride slurry contains small chunks of potassium metal. If the addition of benzyl bromide is not done immediately (within 2 seconds) following when **24** comes in contact with potassium hydride, the expected peach reaction

tint will be replaced by an opaque dark royal blue, and a dark beige solid with a slightly different melting point will form in place of the desired off-white solid. The opaque dark royal blue color is attributed to possible Birch-type reductions, presumably involving single-electron transfer from the valence shell of residual potassium metal within the slurry to the aromatic ring of **24**, forming a radical anion intermediate **48**.[47]

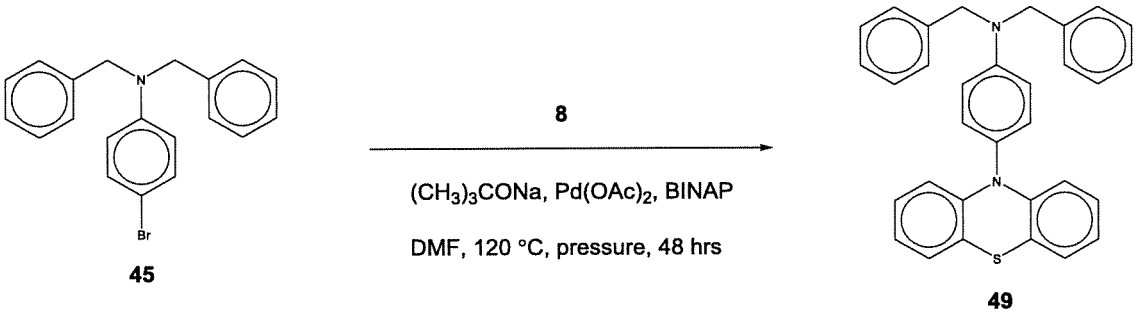


The increased electron-richness of the ring would diminish the conjugated nitrogen atoms ability to function as an electron donor/activator, thereby diminishing its contribution to a resonance hybrid. This, in turn, would make nitrogen less likely to participate as a zwitterion contributor and develop a localized +1 charge (a phenomenon which normally would account for a decrease in the pK_a value of the hydrogen atoms bonded to it). The now-reduced acidity of the N-H bond would further enable single-electron transfer to outcompete the desired N-alkylation.

N-Benzyl-4-bromoaniline **44** exists as an oil at room temperature, and required an extraction work-up for isolation; however, *N*,*N*-dibenzyl-4-bromoaniline **45** is crystalline

and can be isolated by filtration. To obtain **45** in pure yield, dropwise addition of water should be used to neutralize the unreacted potassium hydride, quench the reaction, and precipitate out **45** as an off-white solid. If as little as one additional drop of water is added, **44** will oil out (tiny dark orange droplets will form), re-dissolving **45**, and an extraction work-up will be necessary to isolate **45**. Furthermore, **44** will be mixed with **45**, so chromatography will become necessary. This particular process will provide **45** in greater than 70% yield so long as the amount of starting material **24** used does not exceed an 800 mg scale.

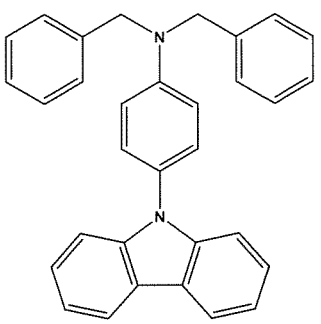
Dibenzyl-protected **45** was now ready to be Buchwald-Hartwig cross-coupled with phenothiazine **8** to prepare **49**, which in itself could serve as a comparison target precursor.



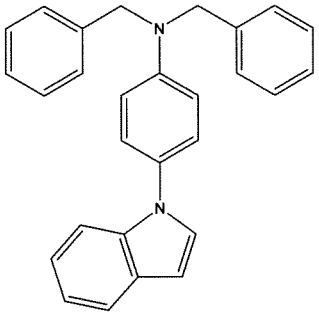
45 **49**

Sodium *tert*-butoxide was used as the base, and triphenyl phosphine was used in place of tri-*tert*-butyl phosphine. $Pd(dba)_2$ was the ligand, and toluene was the solvent. Many attempts were made, with varied concentrations, temperatures, amounts of reagents, and lengths of time before the desired product was formed.

A carbazole analog **50** was prepared by similar methodology; however it was again necessary to repeat experiments with varied conditions before desired product formation occurred.



50



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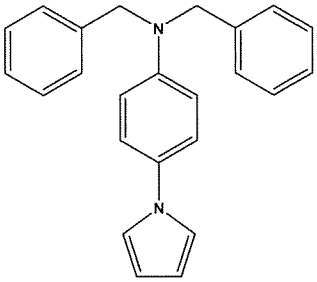


52

Attempts to prepare indole analog **51** and pyrrole analog **52** were unsuccessful under these varied conditions. The methodology was then repeated using BINAP and X-Phos ligands included as part of the suspension, however these conditions were also unsuccessful. Substituting sodium *tert*-butoxide with cesium carbonate also proved unsuccessful.

Filtration of Buchwald-Hartwig reaction mixtures was brought about through a bed of silica gel in a fritted glass funnel to remove inorganic salts. The salts were washed with 5% ethyl acetate/5% dichloromethane/petroleum ether. Various eluent systems were then explored by TLC to determine which solvent system would provide optimal separation of **49** from unreacted **45** by flash chromatography:

Table 1. Comparison of R_f values for *N*,*N*-dibenzyl-4-bromoaniline and *N*,*N*-dibenzyl-4-(10*H*-phenothiazin-10-yl)aniline with various TLC eluting solvents.

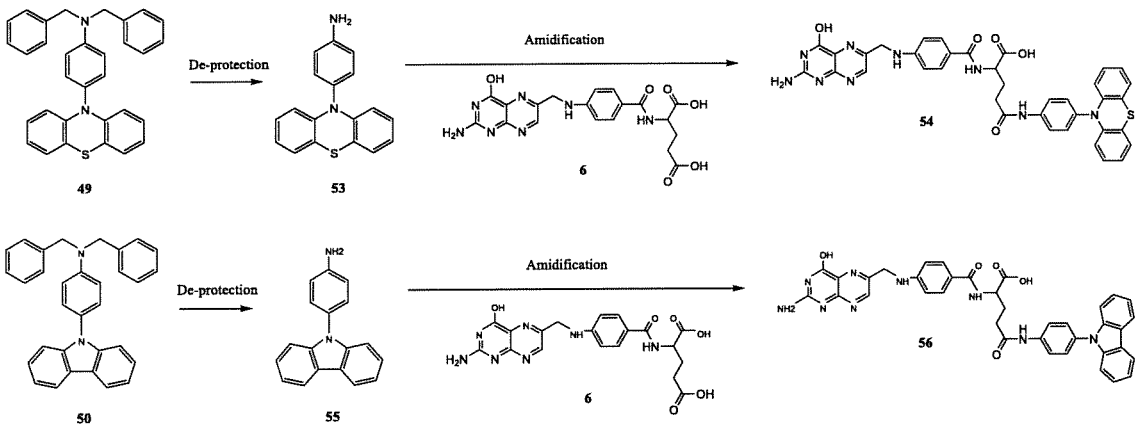
	10% diethyl ether/ petroleum ether	10% toluene/ petroleum ether	50% toluene/ petroleum ether
N,*N*-dibenzyl-4-bromoaniline (**45**)	$R_f = 0.52$	$R_f = 0.03$	$R_f = 0.12$
N,*N*-dibenzyl-4-(10*H*-phenothiazin-10-yl)aniline (**49**)	$R_f = 0.39$	$R_f = 0.13$	$R_f = 0.65$
	R_f difference = 0.13	R_f difference = 0.10	R_f difference = 0.53

The table above supported the use of 50% toluene/petroleum ether as a suitable eluent system for an effective separation of desired product from unreacted organic starting material.

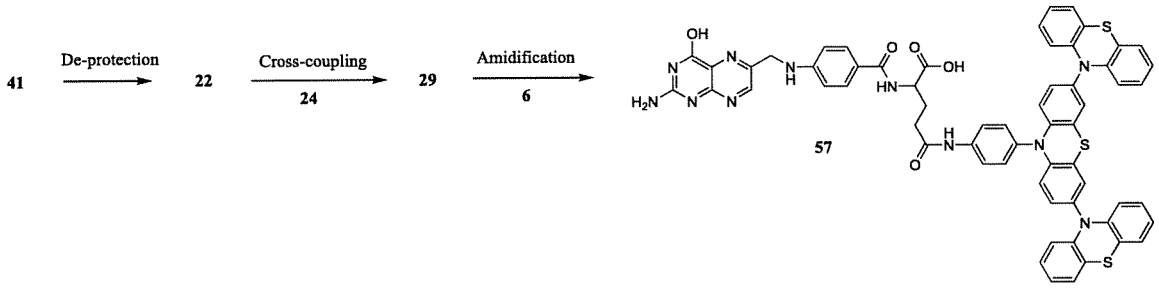
Ullmann methodology was next applied towards making **50**. Dimethyl sulfoxide and *N*,*N*-dimethylformamide were tried (in separate experiments) in place of acetonitrile in an attempt to make the consistency of the suspension closer to that of a solution so that all reagents could react in the same phase; and also so that higher elevated temperatures could be used to promote collision frequency and sufficient activation energy. Nonetheless, no significant difference in yield and purity for crude **50** was noticed between Buchwald-Hartwig and Ullmann methodology. Purification of Ullmann products was brought about by flash column chromatography, with an eluent system consisting of 5% ethyl acetate, 5% dichloromethane, and 90% petroleum ether).

After preparing **39-41** and **49-50**, the next phase of the project was to explore benzyl deprotection. Conversion of **49** and **50** into **53** and **55**, respectively, would produce two different sensitizer-containing compounds, both cross-coupled and pi-

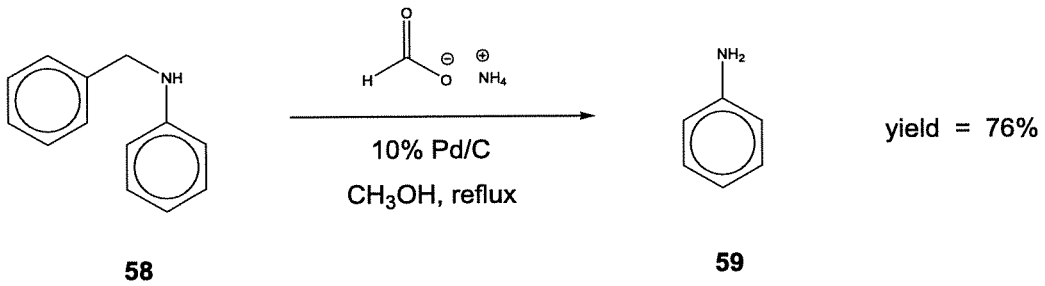
conjugated with an aniline linker, and both potentially suitable for amidification with folic acid **6** to afford targets **54** and **56**.



Additionally, deprotection of **41** would prepare trimer **22**, and subsequent cross-coupling with **24** to produce **29**, followed by amidification with **6**, would produce **57**.

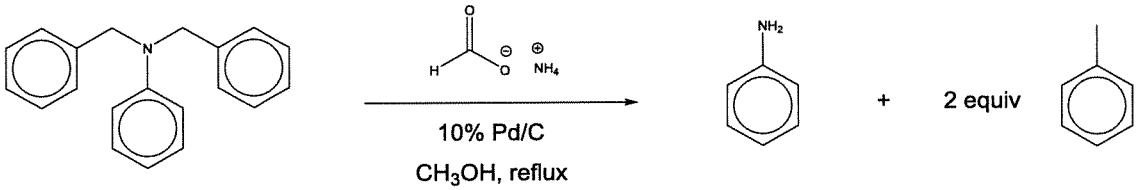


Initial deprotection efforts were employed using a methodology reported by Ram *et al.*[48]



Ram's conditions were applied to previously prepared starting material **50**, but no reaction occurred. The percentage of palladium on charcoal was changed from 10% to

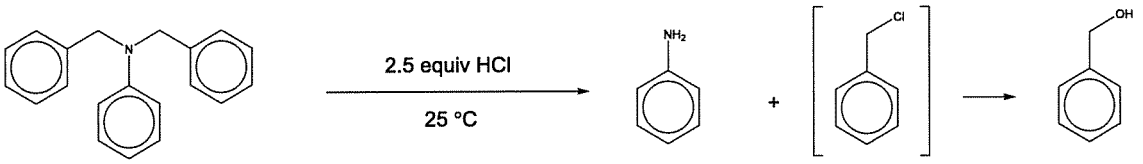
5% but the desired product was not obtained. It was decided that further use of **50** might prove wasteful until a deprotection methodology was developed using a commercially available model. *N*,*N*-dibenzylaniline **60** was selected as the model, as it would liberate aniline **59** upon deprotection, which happens to be the same amine product that Ram's team obtained during their deprotection sequence. Furthermore, aniline is readily identifiable by TLC and by proton NMR. Deprotection of **60** to prepare **59** was attempted using conditions identical to those used on **58**.



60 **59** **61**

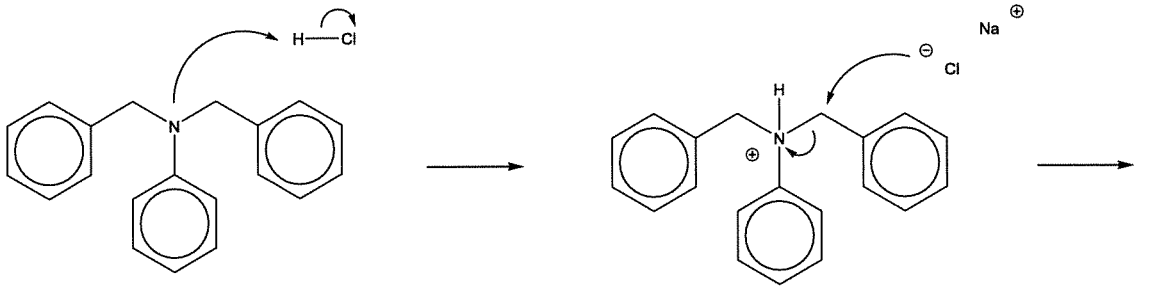
Ammonium formate serves as a proton source for the benzyl-protected nitrogen of **60**, introducing greater polarity to the existing C-N bond, thereby weakening it. Formate anion generates hydride anion upon thermal decarboxylation; which, when protonated, generates hydrogen gas *in situ*. Palladium catalyst on charcoal is insoluble and creates a suspension, but still provides a surface at the solvent interface for which hydrogenation of **60**'s C-N bond could occur. Methanol's polar alcohol group makes ammonium formate soluble, while its non-polar methyl chain introduces enough lipophilicity for **60** to become partially soluble at reflux. Having toluene as a by-product would be advantageous as it is easily recognizable in the proton NMR and also easily removed by vacuum distillation. After several attempts with varied conditions, however, it was clear that **59** and **61** were not formed.

It was envisioned that removal of one or both benzyl protecting groups might be brought about under acidic conditions.

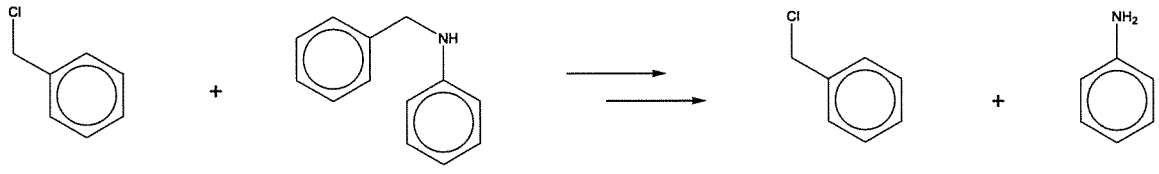


60 **59** **62** **63**

In this strategy, protonation of **60** would activate the nitrogen as a leaving group, with chloride anion attacking the benzyl position of **64**, neutralizing its ammonium ion, forming benzyl chloride **62**, and kicking out **58** in the process. It is noteworthy that in the presence of water, benzyl chloride would be susceptible to hydrolysis to benzyl alcohol **63**. This would be advantageous, as surviving benzyl chloride introduces possible reverse reactions back to mono-protected amines.



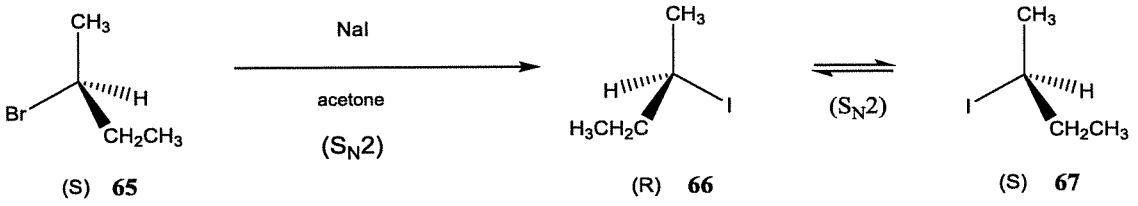
60 **64**



62 **58** **62** **59**

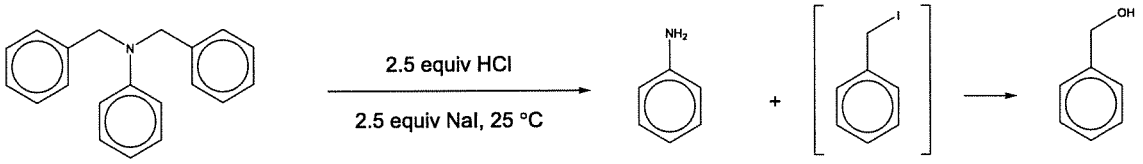
This hydrogen halide deprotection methodology was thoroughly explored. Stoichiometric amounts of HCl were varied at 1.0, 2.5, and excess, with respect to starting **60**. Concentrations of HCl were varied from 3 M to 12 M, with 3 M intended to favor sufficient water content to dissolve chloride anion nucleophile as it formed *in situ* and thereby keeping it in the system, and 12 M intended to favor adequate protonation. Temperatures were varied from 25 °C to 100 °C (reflux). Conditions were repeated under pressure in a pressure tube (120 °C), but without success.

In order to improve upon the effectiveness of the halide nucleophile, it was next envisioned that HCl could be mixed with sodium iodide so as to create Finkelstein-type conditions. A classical Finkelstein reaction involves S_N2 exchange of chloride or bromide leaving groups with iodide. The principle behind the Finkelstein reaction is that sodium iodide remains soluble in acetone while sodium chloride or sodium bromide precipitates out into the surroundings; and by Le Châtelier's principle the conversion of alkyl chloride or alkyl bromide to alkyl iodide proceeds quantitatively. The use of water in place of acetone would keep all halide salts in the system, and given that iodide anion is a better leaving group than chloride or bromide anion, a permanent equilibrium between alkyl chloride/bromide and alkyl iodide could be established. Furthermore, chiral centers at the halide leaving group position would be susceptible to racemization, as repeated S_N2 inversions resulting from sodium iodide reagent reacting preferentially with alkyl iodide product over unreacted starting alkyl chloride/bromide would occur (*e.g.* the 2-bromobutane system).[49]



(S) **65** (R) **66** (S) **67**

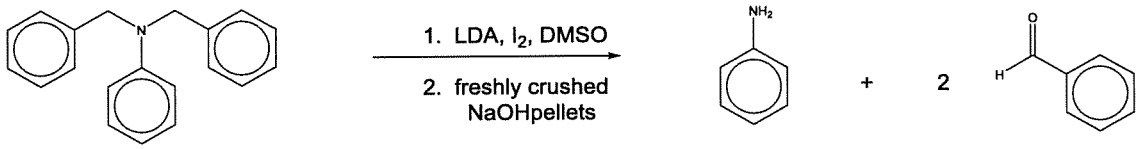
Both our benzyl protected aniline model system and our actual benzyl protected phenothiazine (or carbazole) photosensitizer are achiral and thus, not sensitive to racemization. Whether benzyl chloride versus benzyl iodide is forming as a by-product will not alter which deprotected amine will form, so having a water-based equilibrium instead of an acetone-based Le Châtelier's system will not matter.



60 **59** **68** **63**

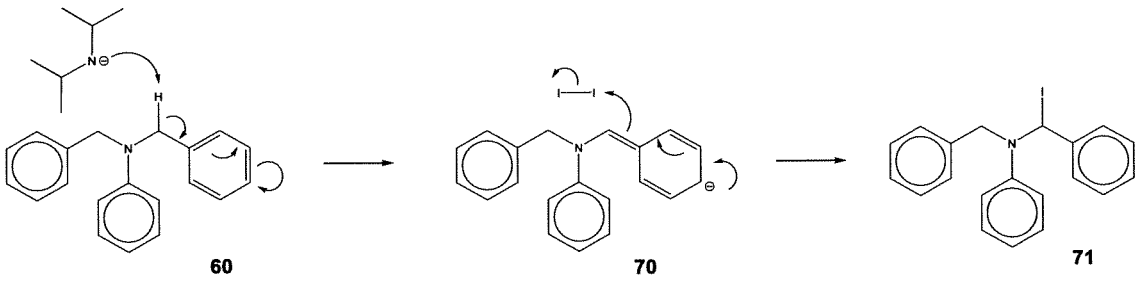
Previous variations in stoichiometric equivalents, concentrations, temperatures, and pressures were tried with the addition of sodium iodide, none of which proved effective. Acetone was introduced one-to-one with water but was also ineffective. Further attempts to use acetone under these conditions were abandoned, as acid-catalyzed self-aldol reactions of acetone monomers to form polymers might dominate. In a final attempt, refluxing with excess concentrated (12 M) HCl mixed with sodium iodide under pressure at 120 °C was employed for 336 hours (14 days), with zero deprotection. It is noteworthy that these harsh conditions, although ineffective towards benzyl C-N bond dissociation, did manage to produce thin flakes of etched glass in the reaction mixture, with permanent rainbows in the pressure tube walls where the glass was etched.

The basic nature of amines makes them inherently poor leaving groups, and base-catalyzed deprotection by substitution seemed counter-intuitive; however, a non-obvious base-catalyzed elimination was envisioned.



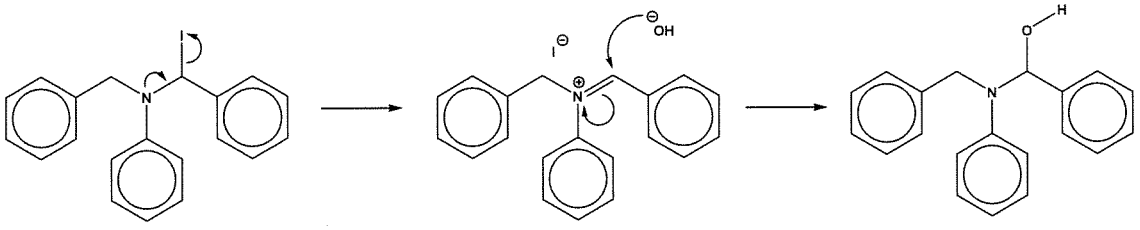
60 **59** **69**

In this strategy, deprotonation of **60** would activate the benzylic position as a resonance-stabilized, conjugate-base nucleophile, and in the presence of diatomic iodide, benzylic halogenation might occur. K_a and pK_a data for benzylamines were not found; however, diisopropylamine's pK_a was reported as 40.0, suggesting that lithium diisopropylamide (LDA) might be a sufficiently strong enough base to remove a benzyl proton. Furthermore, LDA introduces steric hindrance, minimizing its likelihood of replacing iodine in an unwanted S_N1 or S_N2 transformation.



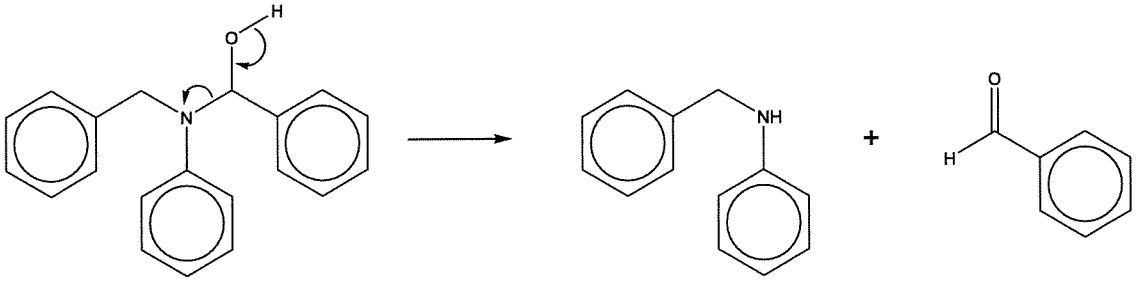
60 **70** **71**

The resulting benzyl iodide **71** would then either survive, or it would undergo self-elimination to produce iminium iodide salt **72**. Either of these intermediates (**71** or **72**), when introduced to aqueous hydroxide in a subsequent step, could undergo hydrolysis at the benzyl position to produce hemiaminal **73**.



71 72 73

Hemiaminal **73** could then quickly collapse to deprotect the amine, forming **58**, and in the process also produce benzaldehyde **69**.

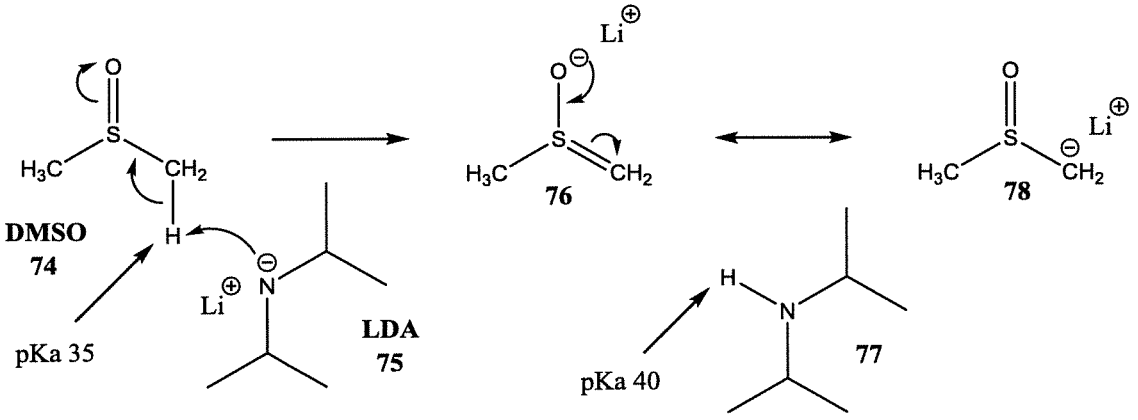


73 58 69

Benzaldehyde would be easily recognizable by its characteristic proton NMR signal at approximately 10.0 ppm. This would not only enable evidence of product formation, but also support for the proposed mechanism by which product formation was occurring.

This methodology was first explored using dimethyl sulfoxide (DMSO, **74**) for the solvent. Compound **74** contains two non-polar methyl groups to assist with the solubility of the carbocyclic components of **60**, and also a polar sulfoxide (S=O) functionality to assist with the solubility of inorganic reagents (Note: NaOH is reported to have a solubility of 35 mg in 100 mL DMSO).[50] Variation in molar equivalents, concentrations, and temperatures, were evoked, but no evidence of benzaldehyde formation was observed.

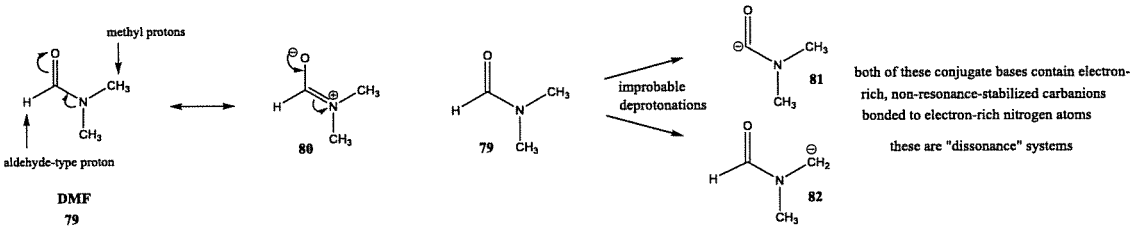
It is noteworthy that the methyl group protons of DMSO, although aprotic, would, upon removal, give rise to a resonance-stabilized conjugate base. Further review of the literature revealed that DMSO's proton exhibit a pK_a of 35, in comparison with the NH of diisopropylamine (the conjugate acid of LDA), which exhibits a pK_a of 40.[51] These values imply that LDA is capable of deprotonating DMSO, which suggests the possibility that deprotonation of DMSO could be outcompeting deprotonation of **60**.



Given that pK_a values are known to be solvent dependent, it was thought that another polar aprotic solvent might be used in place of DMSO.

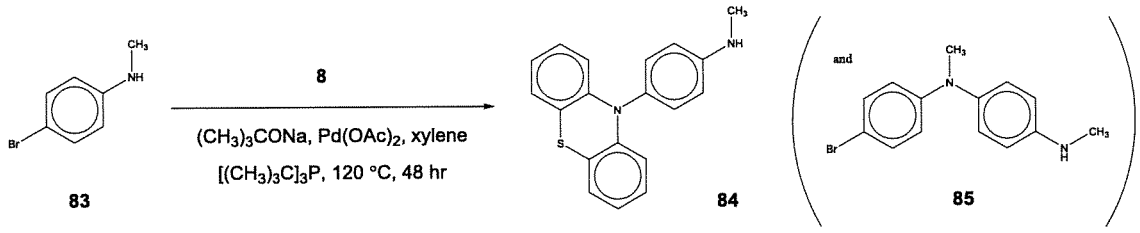
N,*N*-Dimethylformamide (**79**, DMF), like DMSO, is polar aprotic, and should be capable of dissolving hydroxide. None of DMF's methyl protons, if removed, however, would generate a resonance-stabilized conjugate base. Furthermore, the non-bonding electron pair of the nitrogen atom belonging to DMF is delocalized toward the carbonyl, making the carbonyl carbon more electron-rich, and thus, less susceptible to developing an anion via deprotonation of its formyl hydrogen. Thus, use of DMF in place of DMSO would circumvent the previously discussed limitation of using LDA with this system. Although pK_a values for DMF were not found, it is predicted (based on known pK_a

values for standard methyl group protons and aldehyde protons) that its protons exhibit pK_a values similar to those of alkanes (pK_a >56).[52]

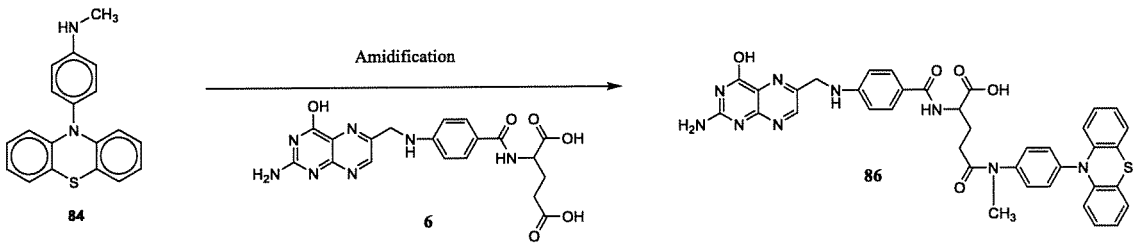


The same variations in molar equivalents, concentrations, and temperatures were invoked as before, with DMF as the solvent; however evidence of benzaldehyde formation was again not observed.

In an effort to circumvent the need for a protection-deprotection sequence, a slightly modified linker, *N*-methyl-4-bromoaniline **83**, was envisioned. The methyl group on the nitrogen provides tetrahedral geometry to the mostly planar environment. It was thought that this three-dimensional component might provide enough steric hindrance to significantly slow the rate of self-cross-couplings by **83**, thereby preventing formation of **85**.

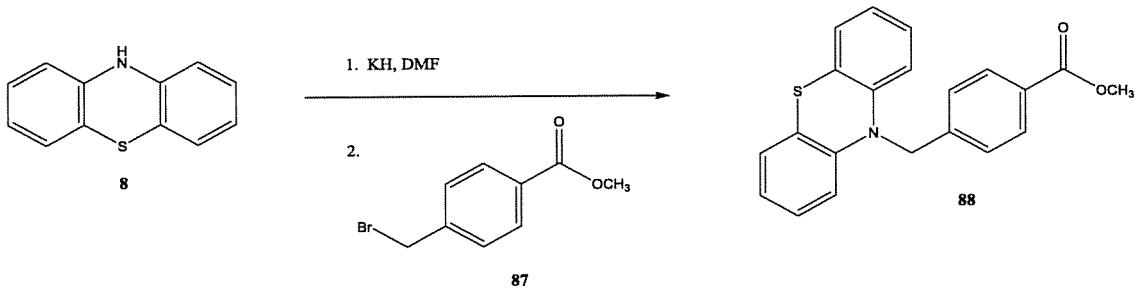


Furthermore, it was thought that the methyl group would not provide so much steric hindrance as to prevent desired subsequent amidification of **84** with folic acid.

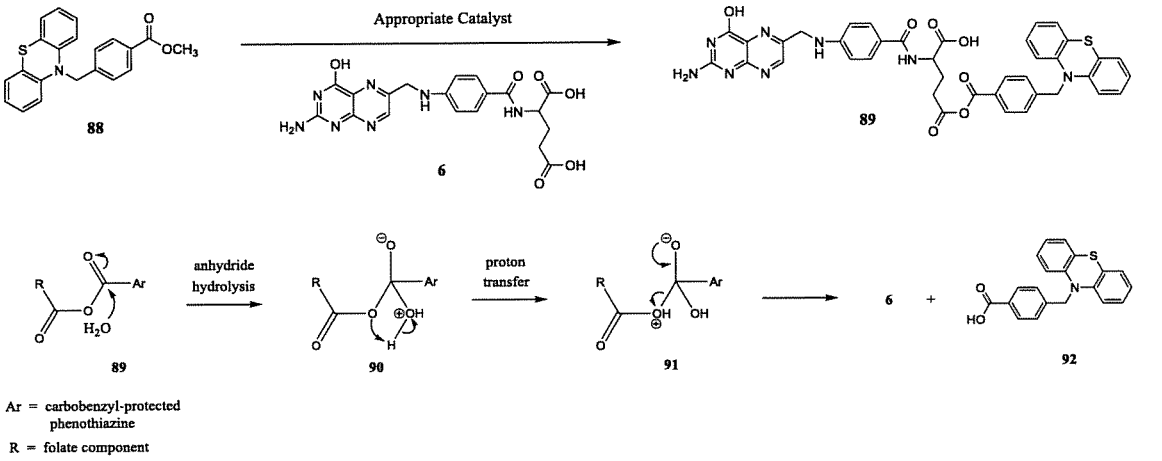


After multiple attempts with varied conditions, neither **84** or **85** were produced, with unreacted starting material **83** being recovered in each case. These results are more attributed to inconsistencies from one Buchwald-Hartwig/Ullmann system's required reaction conditions to the next, rather than attributed to sterics, as other *N*-methylaniline-type systems have been reported to participate in cross-coupling reactions.[53]

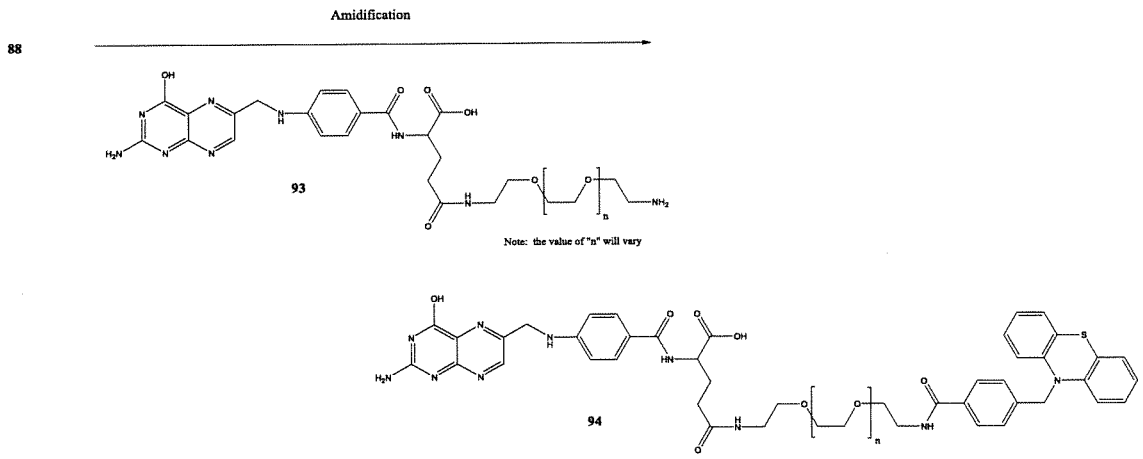
In lieu of not reaching targets **54**, **56**, and **86**, new targets were proposed. The use of a carbomethoxy ester-benzyl protecting group was suggested, as it would circumvent the challenges already faced with both the Buchwald-Hartwig coupling and its subsequent benzyl deprotection.



A direct coupling of the carbomethoxy-protected phenothiazine and the gamma carboxylic acid of folic acid would produce an anhydride functionality (e.g., **89**) which would not survive in an aqueous cellular environment long enough for PDT to be applicable. A more durable functional group would be necessary.

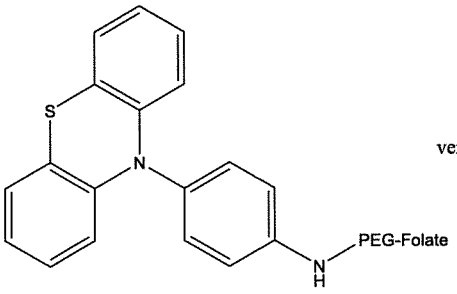


It has been reported that long-chain water soluble polyethylene glycol (PEG)-amines are robust to intracellular environments, and show improved duration with PDT studies.[54] These PEG-amines vary in their molecular masses (2,000, 3,400, 10,000, 20,000, etc.) and are commercially available both as free diamines and also as mono amines with the second amide bonded to folate at its gamma position (e.g., **93**). These PEG-amines can be linked with esters by amidification to form peptide linkages.



The resonance-deactivated carbonyl of the amide functionality (as previously depicted with DMF) makes it one of the least reactive functional groups, and thus, resistant to hydrolysis.

S_N2 transformations between aryl amines and benzyl halides are known to occur readily, and thus, render this approach to be an attractive means by which targets can be obtained. It should be noted, however, that the installation of a sp^3-hybridized benzylic carbon will disrupt conjugation between the pi-system of the photosensitizer and that of the linker, lessening the potential for bathochromic shifts of visible absorbance relative to what **54**, **56**, and **86** might have provided.



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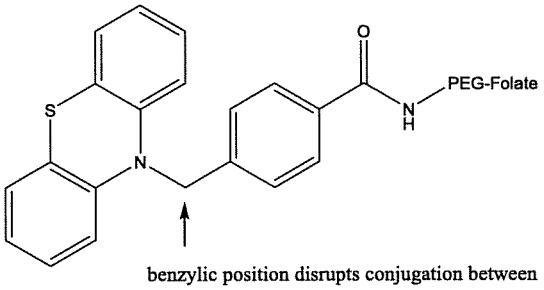


95

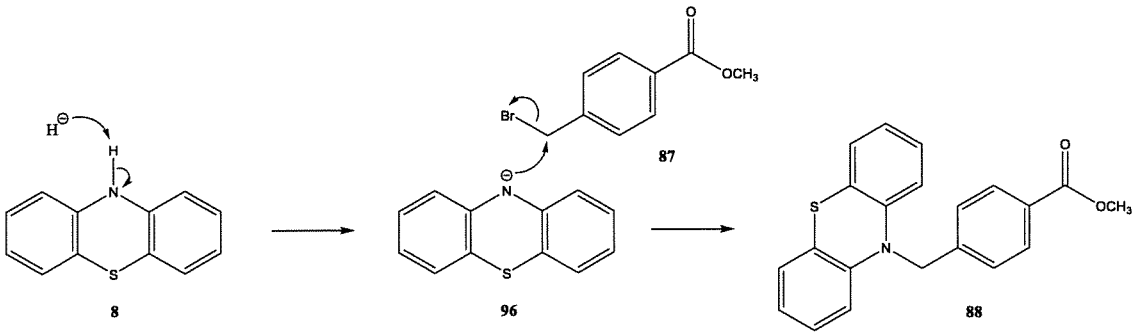
94

benzylic position disrupts conjugation between photosensitizer and linker (nitrogen atom of phenothiazine cannot delocalize electrons within benzamide aryl system)

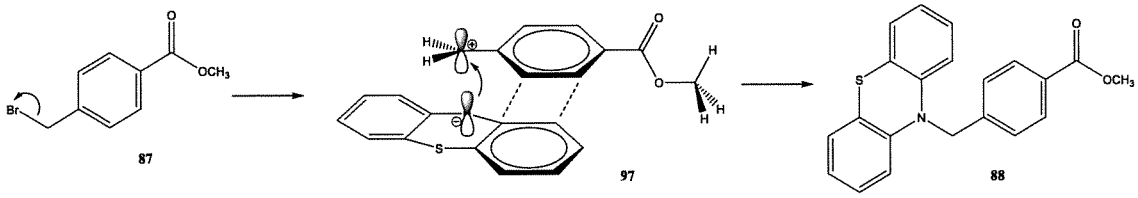
The primary objective of this project was to synthesize molecules that would elicit improved PDT characteristics. Selecting targets on the basis of convenience and/or consistency, with respect to their preparation, is a secondary consideration. These alternate targets, however, with their enhanced water-soluble PEG moiety, still offer potential to improve upon pharmaceuticals already in use.

To initiate the carbomethoxy ester-benzyl protection sequence, potassium hydride is again used to deprotonate the nitrogen atom of **8** in order to make it a more active nucleophile. Hydrogen gas is generated as a by-product. The subsequent bonding for the substitution between the phenothiazine amine anion and the relatively non-hindered benzyl halide of **87** would normally be expected to occur through a bimolecular rate-

determining-step, as a unimolecular pathway would require relatively slow formation of a carbocation sextet, albeit resonance stabilized.

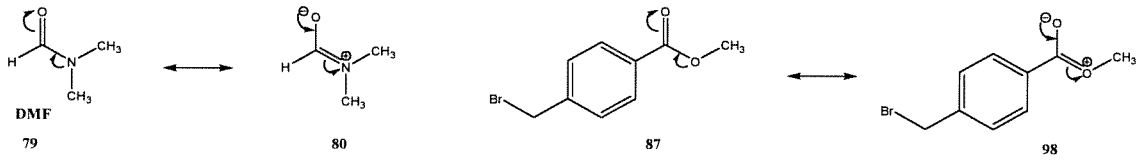


It is noteworthy, however, that this nucleophilic attack is reported to occur through use of p-orbital electrons, rather than through use of non-bonding sp^2 orbital electrons.[55] Pi stacking between the planar phenothiazine nucleophile and the planar benzene ring of the electrophile may favor alignment and overlap of nitrogen's electron-filled unhybridized p-orbital with carbon's electron-vacant unhybridized p-orbital, thereby supporting a unimolecular rate-determining step.



The rate of nucleophilic attack at the carbonyl of **87** is significantly slower, and not observed, in comparison with its benzylic position. Ester carbonyls, like amide carbonyls, are sigma bonded to a heteroatom capable of delocalizing non-bonding electrons into the carbonyl's pi system. Although oxygen's relatively high electronegativity makes it a weaker electron donor, in comparison with nitrogen, a

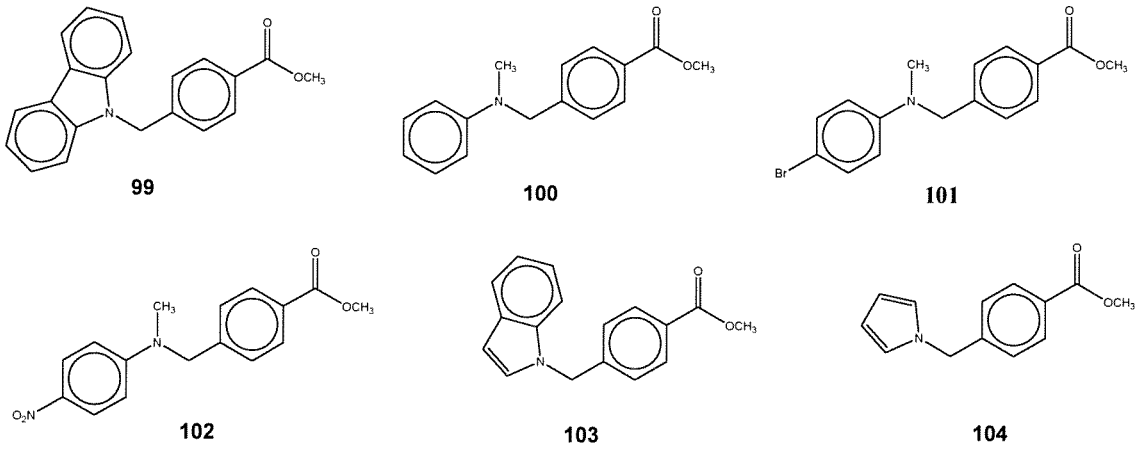
zwitterion contributor analogous to that previously discussed for DMF can form, deactivating the electrophilicity of the ester carbonyl.



DMF

79 80 87 98

This carbomethoxy ester-benzyl protection methodology was further employed with nucleophilic carbazole in an effort to prepare several carbazole-based analogs of **88**.



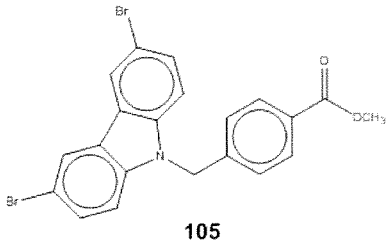
99 100 101

102 103 104

Preparations of analogs **99** (carbazole), **100** (*N*-methylaniline), **101** (*N*-methyl-4-bromoaniline), and **102** (*N*-methyl-4-nitroaniline) were successful, with little need for variance in conditions. Indole analog **103** and pyrrole analog **104**; however, did not appear to be present in their respective product mixtures. It is noteworthy that the nitro group makes **102** unique among the successfully prepared analogs, in that it significantly deactivates its aryl system. Benzene exhibits a maximum absorption at 217 nm in the UV region, whereas nitrobenzene exhibits a maximum absorption at 240 nm. Furthermore, 4-nitrophenol, bearing an activator and a deactivator para to one another, exhibits a maximum absorption at 320 nm, and 4-nitrophenolate anion exhibits a maximum of 400

nm.[56] The tertiary nitrogen in **102**, although incapable of undergoing deprotonation, is a pronounced activator in comparison with a phenolic OH group. These patterns suggest that **102** may exhibit sufficient wavelength to compensate for the previously discussed disruption in conjugation present at each benzylic position among the analogs.

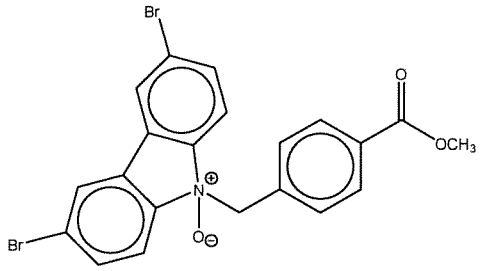
Analog, **99**, was prepared in several batches (all of which afforded similar yield and purity) for the purpose of continuing multi-step synthesis towards PEG-folate conjugate targets. The dibromination methodology previously demonstrated on phenothiazine **8** and on *N*-benzylphenothiazine **40** was successfully employed with **99**, in an effort to prepare a new analog **105** that could (1) provide additional bathochromic shifting in the UV for the eventual PEG-folate conjugate, and (2) serve as a trimer precursor, should Buchwald-Hartwig and/or Ullmann studies be resumed.



105

Analog **105**, like **99**, was prepared in several batches, with consistent yield and purity.

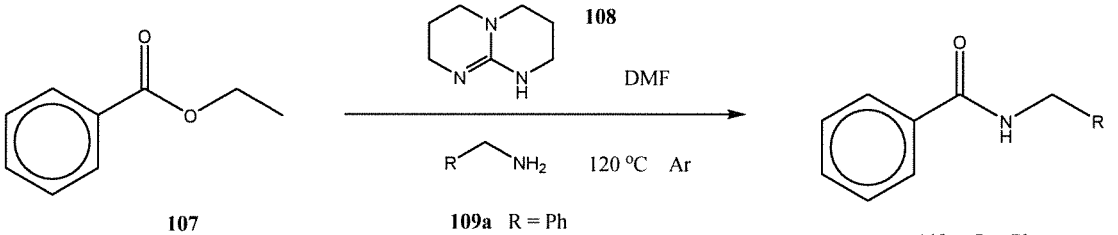
In an attempt to further extend UV activity, **105**, was dissolved in deuterated chloroform and a stream of air was bubbled into solution for 60 min with the intention of preparing N-oxide **106**.[57]



106

NMR analysis revealed that the proton chemical shifts, including at the benzylic position, remained unchanged. The duration of air bubbling was extended to several hours, with occasional $CDCl_3$ re-dilution to prevent unwanted reaction concentration resulting from the bubbling process; however, no spectroscopic change was observed.

Amidification of **99** or **105** with PEG-folate **93** was the remaining step towards preparation of the first conjugate target. Given that **93** was commercially available at approximately $350/50 mg, it was decided that an amidification model system should first be developed. Ethyl benzoate **107** was used to mimic **99**. Benzylamine **109a** and octadecylamine **109b** were both used to mimic PEG-folate.

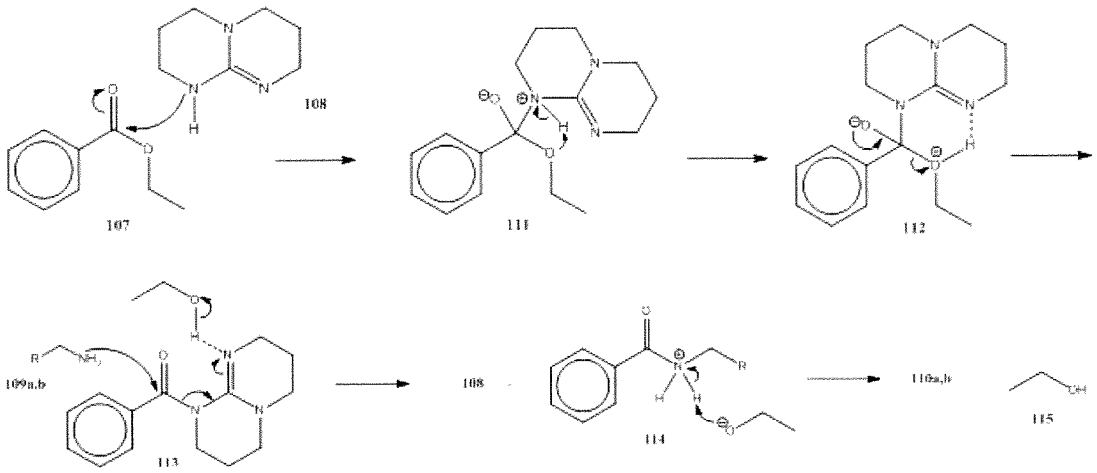


107

109a R = Ph
109b R = $(CH_2)_{16}CH_3$

110a R = Ph
110b R = $(CH_2)_{16}CH_3$

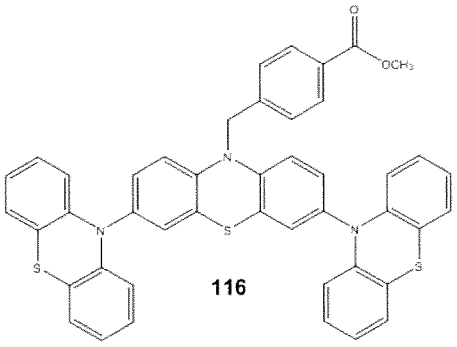
1,5,7-Triazabicyclo[4.4.0]dec-5-ene (**108,** TBD) functions as a bifunctional catalyst, and, as it is not consumed during the course of the transformation, can be used in catalytic amounts.[58]



In both cases, product was not formed under these conditions. This is attributed to the reaction unintentionally concentrating while being maintained at 120 °C for several hours. Ethyl benzoate and DMF, although both significantly high in boiling point (211 °C and 153 °C, respectively), were being used in microliter quantities. Use of a pressure tube did not change this outcome. A 1:1 mixture of DMF/H_2O seemingly increased reagent retention within the system; however ester hydrolysis became competitive with amidification, and again, product formation was not observed.

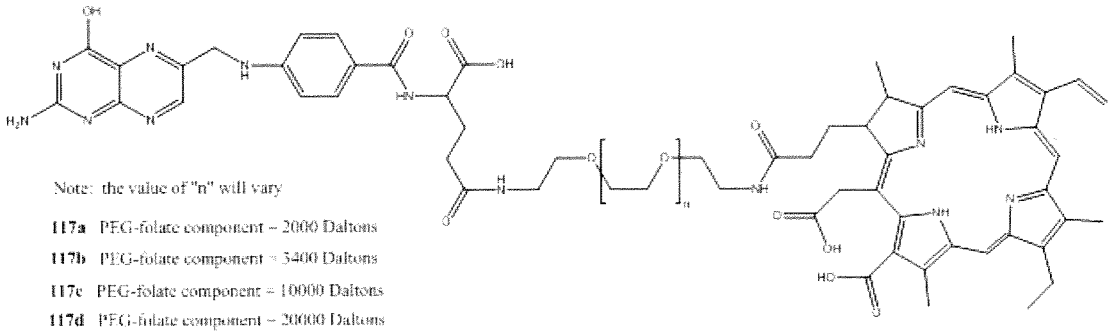
Foreseeing potential problems for costly PEG-amine amidification with carbomethoxy esters, it was anticipated that PEG-amine amidification with a carboxylic acid would be less likely to fail, as acid-amine amidifications have been thoroughly investigated and reported in the literature.[59] Firsthand, hydrolysis or saponification of the carbomethoxy functionality to a carboxylic acid would have to be performed. Ester hydrolysis and ester saponification are known transformations with widespread success; however, UV analysis by Donahue of a highly conjugated carbomethoxy benzyl-

protected phenothiazine trimer **116** revealed a lower-than-expected long wave absorption (<450 nm), leading to the postponement of further carbomethoxy research.



116

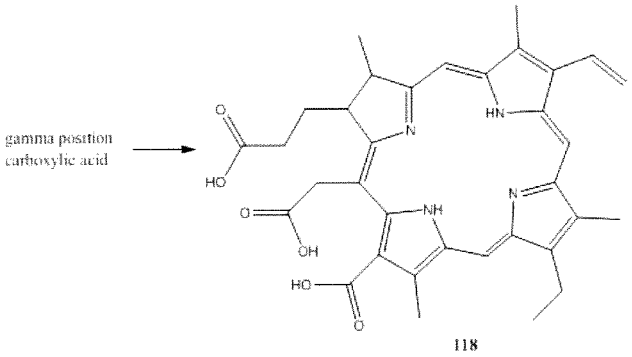
To extend research being conducted, analogs of Donahue's Chlorin-e6 PEG-Folate target **117a** were synthesized.[60] The same methodology was used as by Donahue, except that her 2,000 Dalton polyethylenedioxy (PEG) –folate amine linker was substituted with PEG-folate amines of 3,400, 10,000, and 20,000 Daltons.



Note: the value of "n" will vary

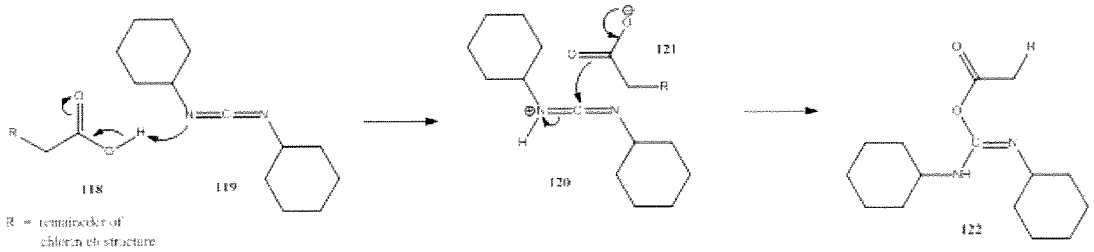
117a PEG-folate component = 2000 Daltons
117b PEG-folate component = 3400 Daltons
117c PEG-folate component = 10000 Daltons
117d PEG-folate component = 20000 Daltons

Chlorin-e6 **118**, a known photosensitizer, is a triacid, and can be linked at the gamma-carboxylic acid position by amidification.

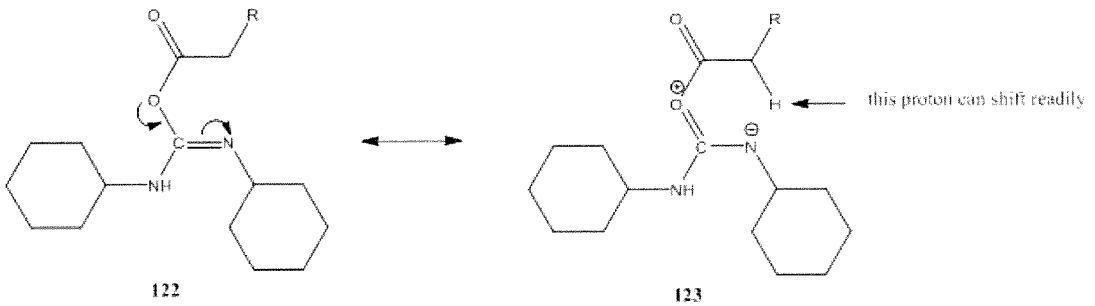


118

Although the alpha and beta acids should also be reactive the amine nucleophile encounters greater hindrance in their vicinity, which slows the rate of their amidification. Even at the gamma position, the reaction is sluggish in the absence of dicyclohexylcarbodiimide (**119**, DCC) coupling reagent. Initially, protonation of one of the DCC nitrogen atoms occurs from one of chlorin-e6's carboxylic acids. The resonance stabilized iminium cation of DCC that forms can subsequently be trapped by the less-hindered gamma carboxylic acid's oxygen atom to form **122**.

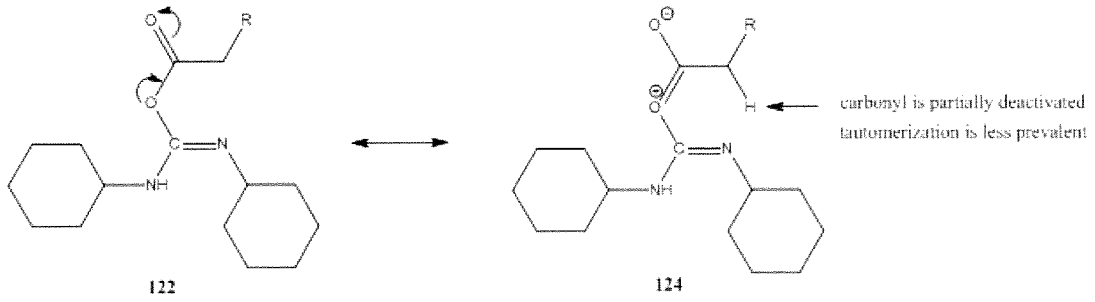


The oxygen in **122** that is flanked by two pi bonds can participate in two separate zwitterion contributors to the overall resonance hybrid. One of these contributors, **123**, involves a nitrogen atom, and does not deactivate the carbonyl's tendency to participate in enol-keto tautomerization.



122 123

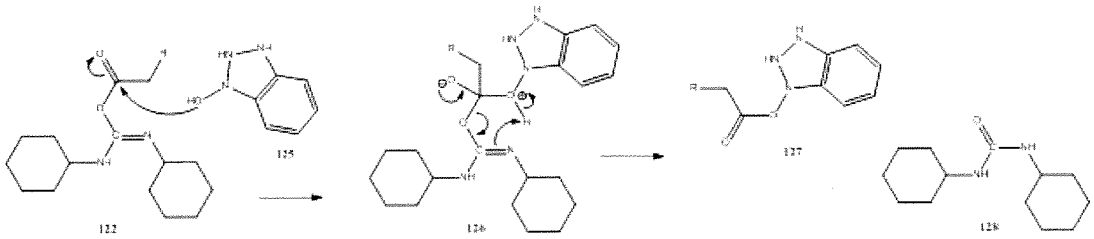
The other contributor, **124**, involves deactivation of the carbonyl, and thereby diminishes the tendency for tautomerization to occur.



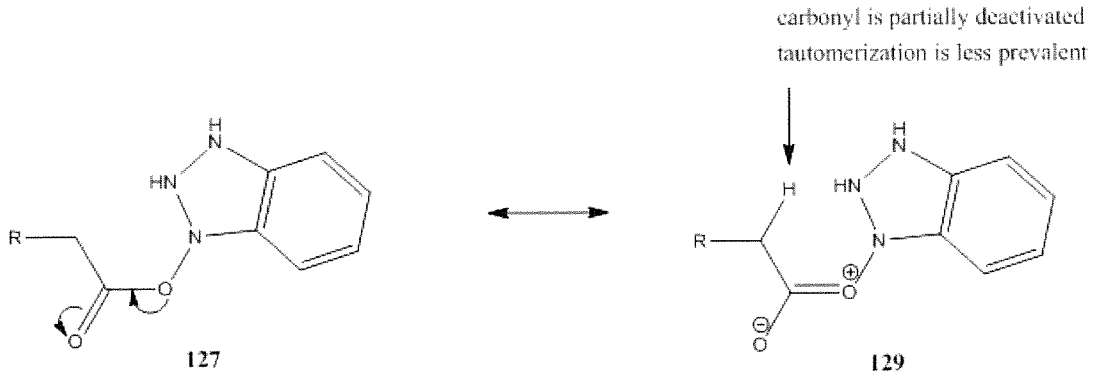
122 124

It is noteworthy (when preparing future analogs) that enol-keto tautomerization changes the hybridization of the alpha carbon from sp^3 to sp^2, thereby changing its geometry from tetrahedral to trigonal planar, and eliminating whatever Cahn-Ingold-Prelog (R/S) stereochemical designation might be present. Had our particular analog been chiral at this position, it would have been susceptible to racemization.

This potential for racemization can be minimized by addition of hydroxybenzotriazole (HOBT) **125**. When added, HOBT exchanges with the DCC-folate adduct in trans-esterification fashion to produce **127** and water-soluble by-product dicyclohexylurea (**128**, DCU).

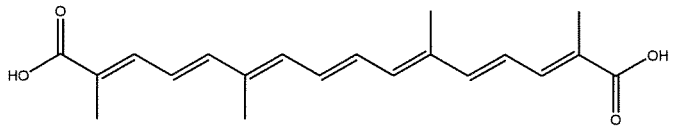


The oxygen atom in **127** that is connected to the carbonyl can only produce one zwitterion contributor, **129**, which is analogous to **124** in that it exhibits a partially deactivated carbonyl. Competition from a non-deactivated-carbonyl zwitterion contributor analogous to **123** is not available, and thus, the tendency for tautomerization remains diminished.



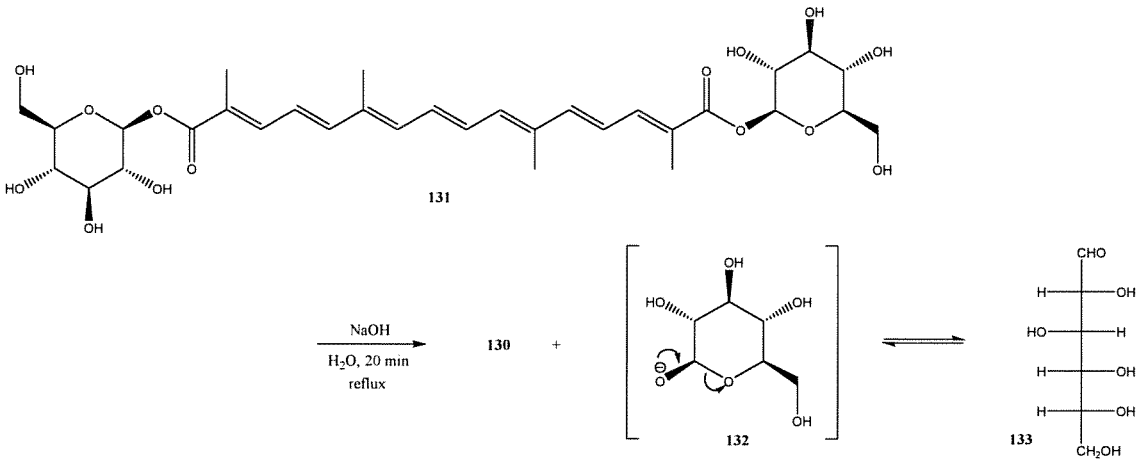
A concurrent objective was to build a new PEG-folate target that would potentially exhibit UV activity beyond Chlorin's 660 nm range. Terpene-containing compounds (e.g., certain vitamins) exhibiting extended pi-conjugation were considered. Crocetin diacid **130** is an olefin-conjugated di-terpene exhibiting trans stereochemistry, making it a potential bathochromic linker. We were surprised to learn that crocetin was already a known anti-cancer compound; but its potential as a PDT sensitizer had not been previously reported.[61] Although hydrophobic in appearance, it was predicted that

inclusion of the 20-carbon crocetin moiety as part of the conjugate would not interfere with the conjugate's water-solubility being supplied by the relatively massive PEG-amine linker.

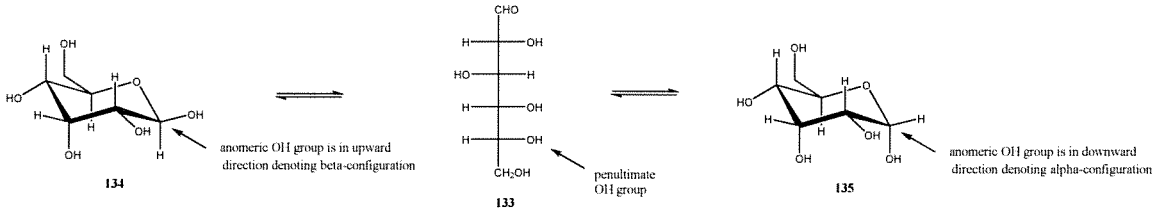


130

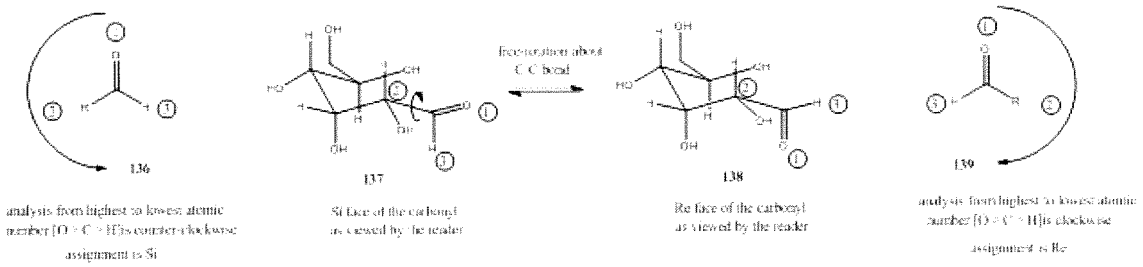
Due to a temporarily unavailability of crocetin **130**, bis(beta-D-glucosyl) crocetin **131** was purchased. Saponification of **131** with excess sodium hydroxide at reflux afforded double acetal deprotection, producing **130** and two equivalents of water-soluble D-glucose **132**.



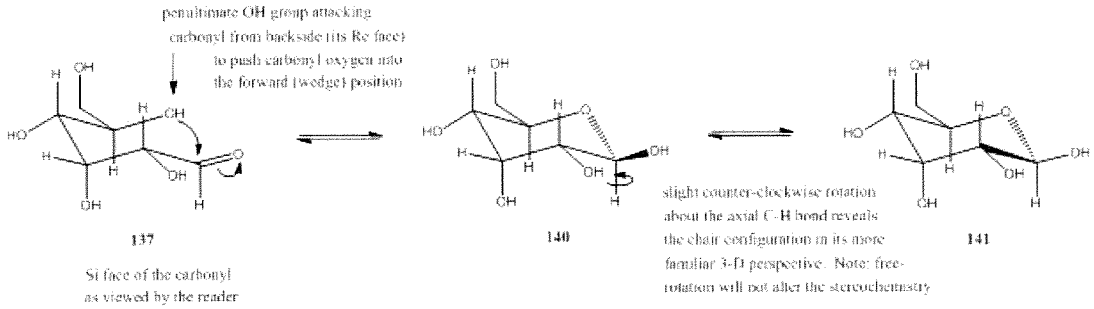
It is noteworthy that **132** can be drawn as a more familiar Haworth projection **134.**



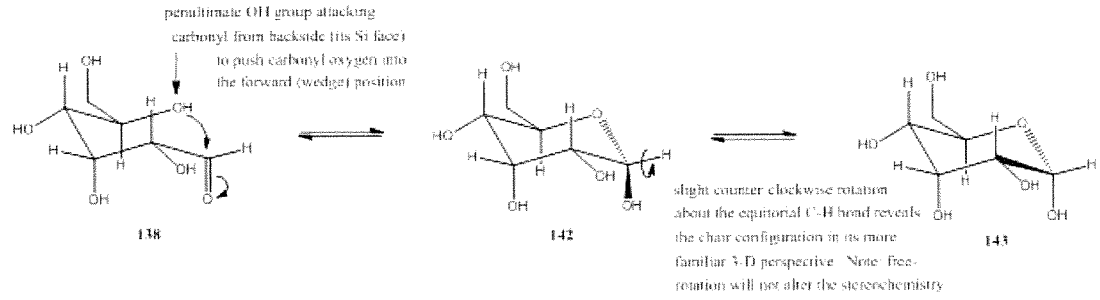
dominate over the Fischer form in aqueous solution. In the open Fischer form, the penultimate (second to last) hydroxyl group of the aldohexose can intramolecularly attack the carbonyl position at either its Re or its Si face. [62]



Intramolecular attack from behind at the Re face of **137** will produce **140**, restoring the original beta configuration observed in **134**.



Free-rotation along the $C_1 - C_2$ bond of **137** reveals the Re face of attack to the viewer (i.e., **138**). Intramolecular attack from behind at the Si face of **138** will produce **142**, creating the alternate alpha configuration observed in **135**.



Interconversion between alpha and beta forms via the Fischer intermediate (referred to as mutarotation) is prevalent among reducing sugars (those that lack a full acetal functionality at their anomeric position.

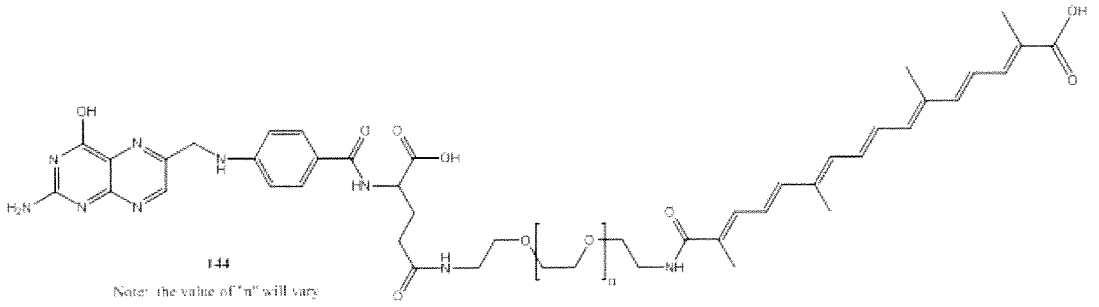
Crocetin **130** is an extremely fine red powder, analogous in texture to charcoal surfaces used in palladium-mounted catalysts. It was found that it could not be filtered, as it imbedded in fritted glass and also in filter paper. Centrifugation was used to compact product **130** into the bottom of the suspension; however, removal of water by pipet was incomplete. Attempts to pipet away more than an estimated 80% of the water resulted in **130** also being drawn into the pipet, even with the pipet flush against the bottom of the tapered centrifuge tube. Proton NMR revealed that **130** had formed; however, even after air drying for several days, a dominant water peak was present, and the percent yield was unobtainable from the proton NMR.

Taking into account the volume of water that the crocetin product contained, lyophilization (a technique capable of removing > 95% of water from a suspension) was thought to be appropriate. During lyophilization, a suspension is initially frozen, and then the aqueous component is removed under vacuum by sublimation, leaving behind the desired solid. Lyophilization on the crocetin suspension, however, could not be done as the equipment was inoperative. Removal of residual water using a drying oven was attempted; however, the color of the powder changed from red to brown (crocetin is known to be red in color), and visible water was still present. Obtaining a melting point of the brown material and comparing it to that of the red material might have enabled determining whether or not oven-drying decomposition had occurred; however, even

trace amounts of water will function as an impurity and cause the experimentally determined melting point to be inaccurate by several degrees. Melting and freezing are phase changes that occur in opposite directions, but the colligative property "freezing point depression" is applicable to both.[63] The presence of an impurity will generally lower, or depress, the experimentally obtained initial melting point and broaden the experimentally obtained melting point range.

Alternatively, protonation of **131** with concentrated hydrochloric acid at elevated temperature brought about a mixture of mono and di-acetal deprotection. The same isolation-from-water limitations that were observed under basic conditions were again observed here.

Crocetin **130** became commercially available, existing as a diacid, however, **130** would be succeptible to di-amidification with the costly PEG amine-folate, unless used in excess. Crocetin (98% purity) sells for $200/g, thus, its usage is meant to be sparing. Given that most transformations afford less than 100% yield, it is preferable to postpone the most costly transformation until the end of a linear multi-step synthesis. This transformation, once accomplished, would mark the end of the multi-step preparation of a crocetin-PEG amine-folate target **144**.



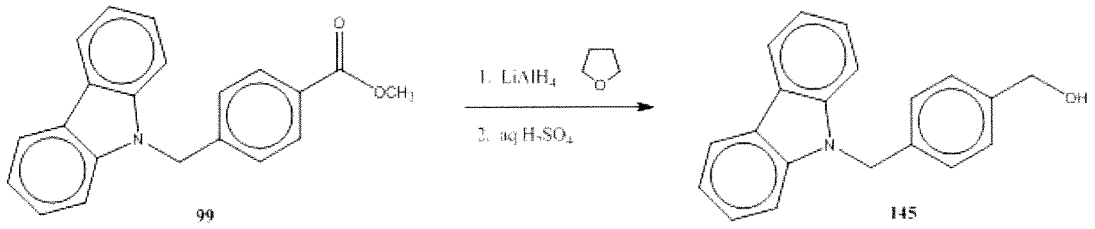
144

Note: the value of "n" will vary

An in-vitro comparison would then allow for toxicity and cell death comparison between known anti-cancer porphyrin chlorin e6 **118**, chlorin e6-PEG amine-folate **117**, known anti-cancer carotenoid crocetin **130**, and crocetin-PEG amine-folate **144**.

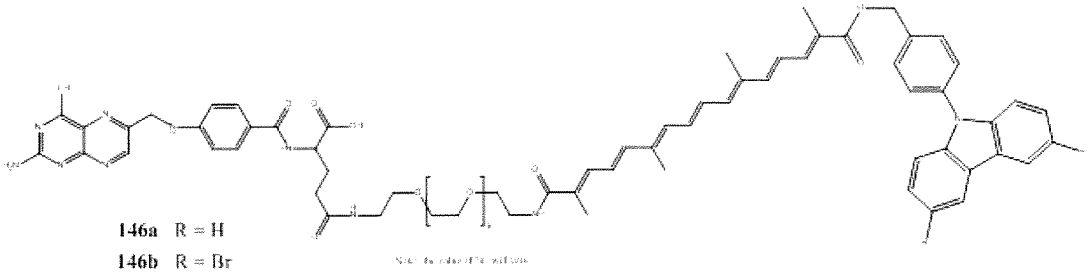
Additionally, **144** could be used as a limiting reagent in a coupling reaction with the relatively inexpensive carbomethoxy benzyl-protected carbazole already prepared in abundance. This would: (1) provide a new target with greater UV wavelength potential, and (2) leave only one end of the diterpene available for amidification, thereby, reducing the stoichiometry to 1:1 for that final transformation. Direct coupling of crocetin diacid with the carbomethoxy-protected carbazole would lend itself to the same limitation as previously discussed for direct coupling of carbomethoxy-protected phenothiazine and the gamma carboxylic acid of folic acid; an aqueous-labile anhydride functionality would form which would not survive in an aqueous cellular environment long enough for PDT to be applicable. A more durable functional group would be necessary.

It was envisioned that reduction of **99** with lithium aluminum hydride (LAH) in tetrahydrofuran (THF) would reduce the carbomethoxy ester to a benzyl alcohol **145**.



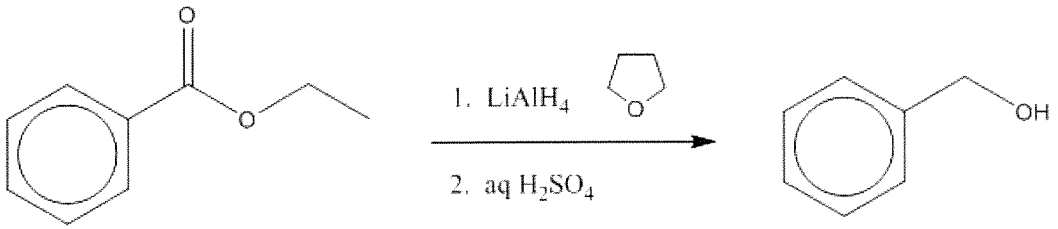
99 145

Alcohols, when reacted with carboxylic acids, form esters, which are robust in comparison with anhydrides. Preparation of **146a,b** would offer a carbazole-crocetin-PEG-amine-folate conjugate as a target that could survive in a cellular environment.



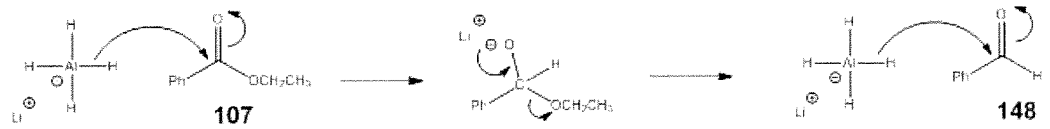
146a R = H
146b R = Br

Rather than consume the inventory of previously prepared **99** during optimization of reduction conditions, commercially available ethyl benzoate **107** was used as a model.

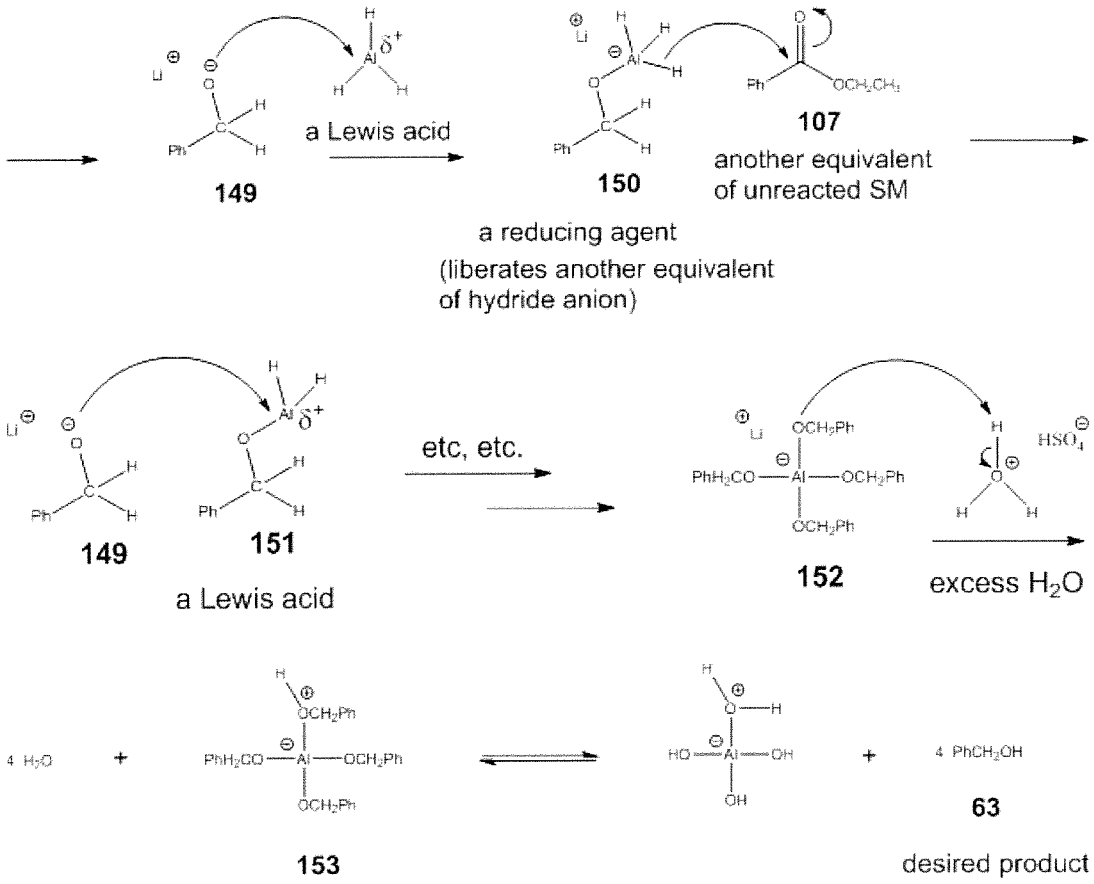


107 **63**

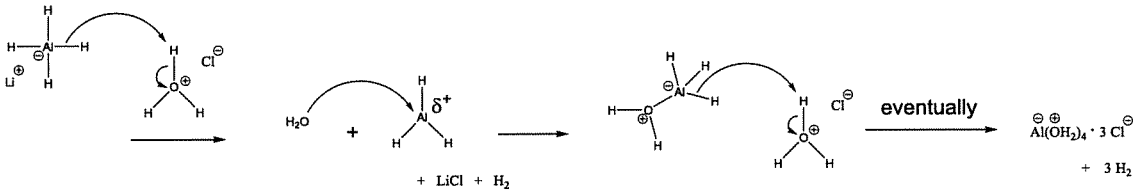
LAH is often referred to as a hydride delivery agent, because it is a source of hydride anion (H^{-1}). The Al – H bond is polarized, with hydrogen being the more electronegative atom and thus, the nucleophilic/basic atom of the reagent. It therefore functions as a hydride anion, and not as a proton. Use of LAH should be conducted with anhydrous ether solvents, such as THF, as they are unreactive with base. It is noteworthy that all four hydrogen atoms of $LiAlH_4$ are available as nucleophiles. The process involves the aluminum atom undergoing oxidation, then reduction, then oxidation, then reduction, etc., until the eventual aqueous hydrolysis work-up step.[64]



107 a reducing agent **147** **148**



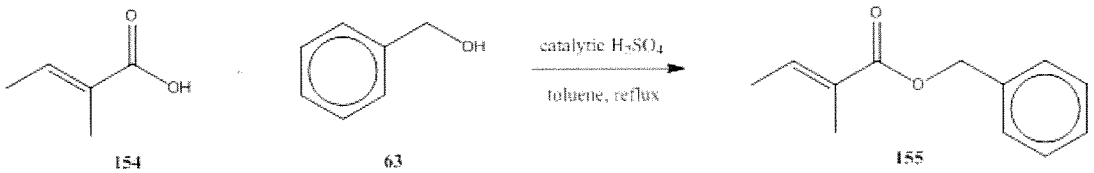
One equivalent of $LiAlH_4$ contains four useful equivalents of hydride anion. Thus, for every 1 mol of ester used, only 0.50 mol of $LiAlH_4$ is required. In addition to liberating the desired alcohol product, the aqueous work-up will also destroy any remaining unreacted $LiAlH_4$ by converting it into hydrogen gas and aluminum hydroxide salts (due to the basic nature of $LiAlH_4$).



Initial attempts to reduce ethyl benzoate did result in formation of desired benzyl alcohol without surviving ethyl benzoate, as revealed by ^{1}H-NMR and by TLC; however, the isolated product was in less than 10% abundance. This is attributed to the formation of water-soluble aluminum-benzoxide complexes that were not isolated during the reaction work-up, albeit the procedure had been taken from the literature: dropwise addition of water, followed by 15% sodium hydroxide, followed by diethyl ether extraction. After drying the ether solution over sodium sulfate, filtration and concentration[65] afforded the desired product, but in lower yields.

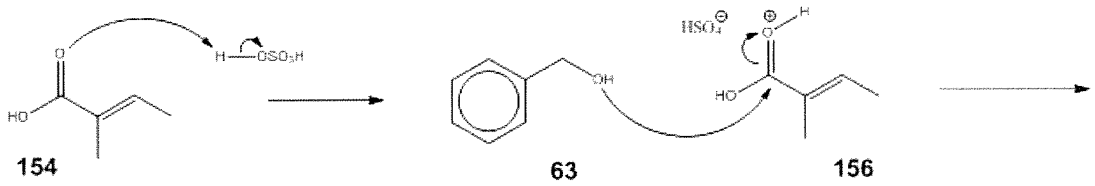
It was then discovered that ammonium chloride has been reported to have been used during aqueous acid work-ups to facilitate the dissociation of aluminum-alkoxide complexes.[66] A repeat of the reduction followed by these new work-up conditions afforded yields of desired product in excess of 60%, both for the model system and for the carbomethoxy benzyl-protected carbazole system.

The next task would be to connect carbazole-protected benzyl alcohol **145** to crocetin diacid **130** by esterification. The model selected was benzyl alcohol **63** as a mimic of the carbazole-protected benzyl alcohol, and tiglic acid **154** as a mimic of crocetin. Fischer esterification conditions afforded greater than 90% yield.

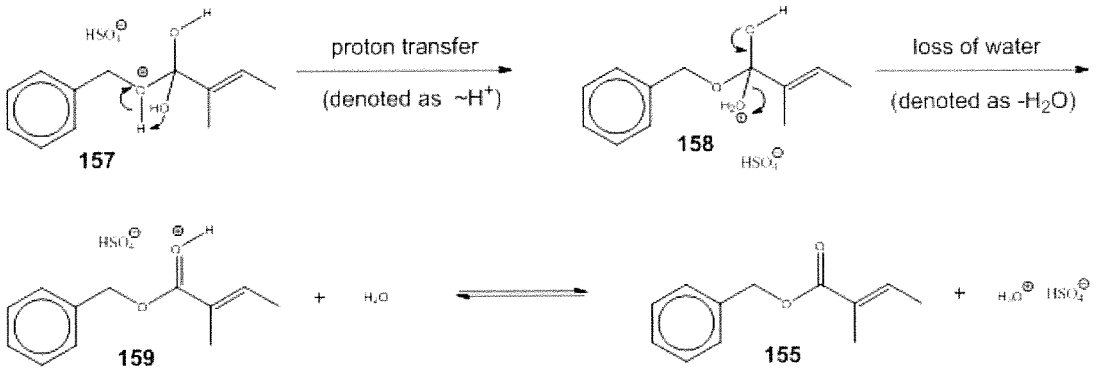


154 63 155

During the Fischer process, catalytic acid protonates the carbonyl, activating it towards nucleophilic attack.

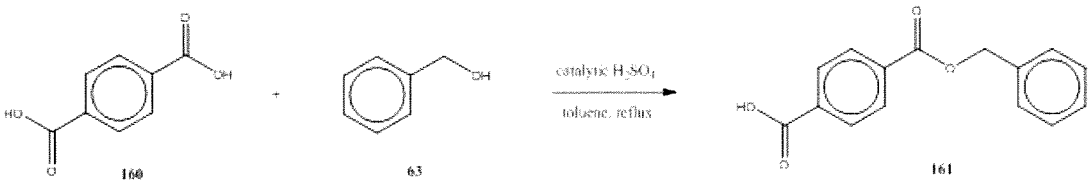


Intramolecular proton transfer generates a water leaving group, and re-formation of the carbonyl produces the protonated ester product **159**.

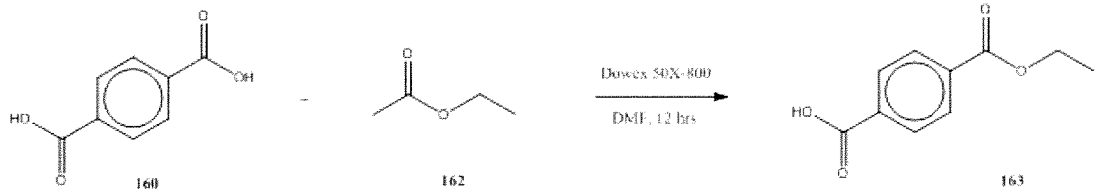


Protonated ester **159** supplies a proton that can be intermolecularly transferred to another equivalent of unreacted **154** in order to repeat the process, hence, allowing the use of sulfuric acid to be catalytic. Mild base is used during the work-up in order to remove the acidic proton so that a neutral ester product can be isolated.

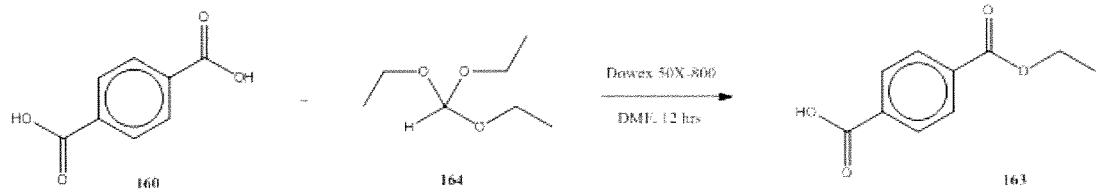
In the event that using crocetin as a nucleophile during Fischer esterification is desired (via reduction of its carboxylic acid functionality to an alcohol), an alternate model was envisioned. Terephthalic acid (benzene-1,4-dioic acid) **160** would serve as a mimic for a hydrolyzed version of the carbomethoxy benzyl-protected carbazole, while benzyl alcohol **63** would again be used as the nucleophile.



The poor solubility of terephthalic acid in both polar and non-polar solvents made for difficult production of **161**. Treatment of **160** was then conducted using Dowex 50X-800 acidic ion-exchange resin in place of sulfuric acid and ethyl acetate **162** in place of benzyl alcohol to prepare **163**. Nonetheless, product **163** was not isolated.



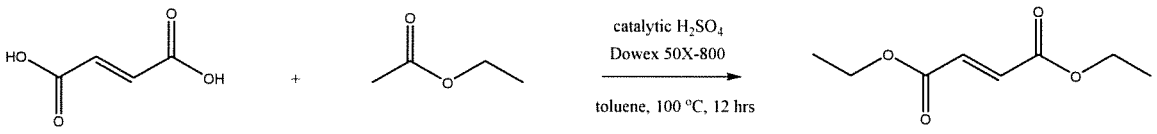
These conditions were repeated using triethyl orthoformate **164** in place of ethyl acetate, again, without formation of **163**.



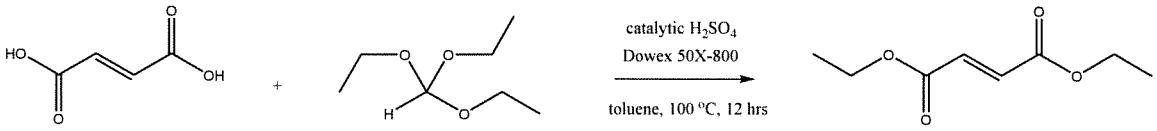
Repeated attempts to obtain **163** using varied solvents (heptane, toluene, xylene), elevated temperature, and the addition of sulfuric acid catalyst in combination with Dowex resin were all ineffective. Even under overnight, robust, continuous extraction conditions, esterification of **160** was unsuccessful. It is noteworthy that obtaining a proton NMR of fresh **160** was inconclusive, as it exhibited poor solubility in $CDCl_3$, d_6-DMSO, and D_2O.

conditions, esterification of **159** was unsuccessful. It is noteworthy that obtaining a proton NMR of fresh **159** was inconclusive, as it exhibited poor solubility in $CDCl_3$, d_6-DMSO, and D_2O.

As a measure of determining whether or not the Dowex resin was still active or if it had been exposed to humidity for too long of a period, reaction conditions were repeated with fumaric acid **165** used in place of terephthalic acid, first with ethyl acetate, and then again with triethyl orthoformate. As an aside, fumaric acid **165** and crocetin diacid **130** are analogous in structure, and thus, fumaric acid served as an additional model.

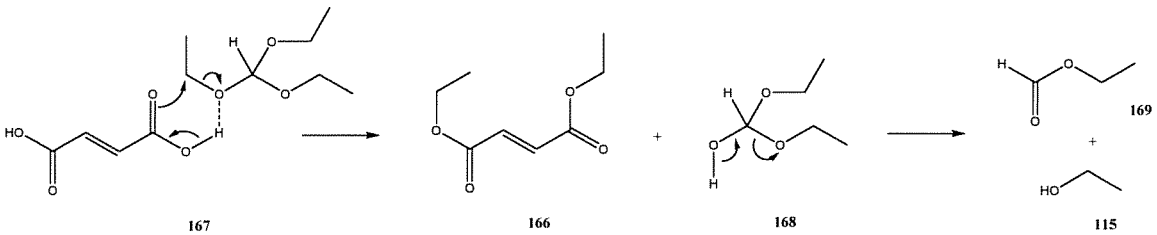


165 **162** **166**



165 **164** **166**

In both circumstances, formation of **166** was observed in approximately 65% yield. In the case of triethyl orthoformate as the supplier of the ethoxy group, ethyl formate and ethanol were also produced.



167 **166** **168** **115**

This led to the conclusion that the Dowex resin was in fact active, and that terephthalic

Further investigation of crocetin and its potential conjugates was postponed in lieu of new data having been obtained in a joint venture between the chemistry department and the biology department at Loyola University. *In vitro* studies of HeLa Cell exposure to the Chlorin e6-PEG 2,000-folate conjugate prepared by L. Donahaue reported oncogenic cell death at 5 micromolar (μM) concentration. Attempting to reproduce Donahue's findings would offer two-fold conformation: (1) the reliability of the methodology; (2) the reproducibility of the data; and an extension to the longer PEG conjugates

To begin this joint venture, it was necessary to calculate the volume of phosphorus buffer saline (PBS) to be added to a given mass of conjugate material available, in order to prepare a stock solution of the desired concentration:

$$\frac{1\text{ mg PEG}_{2000}\text{ conjugate}}{2579\text{ mg}} \left| \frac{1\text{ mmol}}{1\text{ mmol}} \right| \frac{1000\ \mu\text{mol}}{40\ \mu\text{mol}} \left| \frac{1000\text{ mL}}{} \right. = 9.69\text{ mL}$$

The calculation above shows that by taking 1 mg of material and dissolving it in 9.69 mL of phosphate buffer saline (PBS), a 40 μM stock solution was able to be prepared. Prior to introducing the incubated PDT conjugate HeLa cells, it was necessary to measure out multiple 75 μL portions of this 40 μM stock solution for the purpose of adding them to 72 of the 96 wells (all except columns 1, 5, and 12).

Next, 25 μL of the 40 μM solution from each respective well was transferred to a new plate designated for upcoming light exposure. To each of these wells, 175 μL of media was added, bringing the total volume of each well containing conjugate to 200 μL.

As this total volume was now eight times as dilute as what was originally removed from the plate, the original 40 μM concentration was now also eight times as dilute, or 5 μM (the intended concentration for light exposure). Another 25 μL of the 40 μM solution was then transferred from each respective well of the original plate to another new plate designated for dark exposure. Again, to each of these wells, 175 μL of prepared cell media was added, bringing the total volume of each well containing conjugate to 200 μL.

The purpose of having filled the wells of the original plate with 75 μL instead of the required 50 μL was to minimize pipetting error (otherwise, it would be required to remove every drop of material from the well, which is impractical). Generally, eight or more wells are designated as "cell-only controls" and are not exposed to drug. With 88 usable wells multiplied by 75 μL per well equaling 6,600 μL total needed volume, the 9,690 μL prepared stock solution provided adequate volume for one assay.

PEG-Folates are commercially available through NANOCS. Their molecular mass is expressed in Daltons, and reflects the g/mol molecular mass of the sum of the PEG amine linked to folic acid.[67] The PEG-folate 2,000 used by Donahue is 2,000 Daltons (2,000 g/mol). The value "2000," therefore, does not reflect the number of ethyleneoxy monomer units within the structure. Consider folic acid, with a molecular mass of 441 g/mol. Water, the leaving group during amidification, has a molecular mass of 18 g/mol. A single ethyleneoxy [–CH_2–CH_2-O–] unit has a molecular mass of 44 g/mol. Thus, a commercially available PEG-folate 2,000 will contain "approximately" (2000 – 441 – 18)/44 = 35 ethyleneoxy units (not 2,000 units). Note: the Dalton value is based on an average number of ethyleneoxy units, with greater than 80% of the

individual molecules in the NANOCS storage bottle containing 35 ethyleneoxy units, hence the word "approximately." A given PEG-folate 2000 molecule might have as few as 33 ethyleneoxy units or as many as 37 ethyleneoxy units. A table reflecting the number of ethyleneoxy units for a given PEG-folate has been constructed and is provided below:

Table 2. Commercially available PEG-folates, their masses, and their composition.

(PEG-Folate)$_n$	**Number of [–CH_2–CH_2-O–] units**
n = 2000 Daltons	35
n = 3400 Daltons	67
n = 10000 Daltons	217
n = 20000 Daltons	444

Chlorin e6 has a molecular mass of 597 g/mol. Water, the leaving group during amidification, has a molecular mass of 18 g/mol. Thus, a Chlorin e6-PEG-folate conjugate will have a molecular mass of 2000 + 597 – 18 = 2579 g/mol. A table reflecting the molecular mass of prepared conjugates has been constructed and is provided below:

Table 3. Synthetically prepared PEG-folate conjugates and their masses.

Chlorin e6-(PEG-Folate)$_n$ Conjugate	**Molecular Mass**
n = 2,000 Daltons	2,579 g/mol
n = 3,400 Daltons	3,979 g/mol
n = 10,000 Daltons	10,579 g/mol
n = 20,000 Daltons	20,579 g/mol

A table reflecting the volume of PBS to be added by the chemist to a 1 mg sample of conjugate to obtain a specified concentration stock solution has been constructed and is provided below:

Table 4. Volume of PBS required for a given PEG-folate conjugate to obtain a specified stock solution concentration.

PEG units	**40 μM stock soln**	**4 μM stock soln**	**0.8 μM stock soln**	**0.4 μM stock soln**
2000	9.69 mL PBS	96.94 mL PBS	484.68 mL PBS	969.37 mL PBS
3400	6.25 mL PBS	62.55 mL PBS	314.15 mL PBS	628.30 mL PBS
10000	2.36 mL PBS	23.61 mL PBS	118.16 mL PBS	236.32 mL PBS
20000	1.21 mL PBS	12.14 mL PBS	60.74 mL PBS	121.48 mL PBS

When 175 μL of media is added to the 25 μL portions of stock solution already measured into each well, the specified concentrations are produced. A table reflecting these specified concentrations of drug that the cells are actually introduced to during the assay has been constructed and is provided below:

Table 5. Specified concentration of drug for a given assay concentration.

40 μM stock soln	**4 μM stock soln**	**0.8 μM stock soln**	**0.4 μM stock soln**
5 μM assay	0.5 μM assay	0.1 μM assay	0.05 μM assay

A complete listing of the duties for the cell killing studies was shared by K. Kiernan (Department of Biology, Loyola University Chicago), and is provided below:

The first duty is the thawing out of new cells. The hood was sterilized with ethanol and the glass vacuum pipette tip was changed. HepG2 and HeLa cells are currently located in cryotubes in the -80 freezer (second floor common equipment room, Life Sciences Building, Loyola University Chicago). Each tube contains approximately 1 mL of liquid. The cryotubes should be thawed in a 37 degree water bath. The complete media should also be warmed in a 37 °C water bath.

Using a 1000 μL pipette, the contents were drawn up and down to re-suspend the cells, then transferred to a 15 mL Falcon tube. The cells were centrifuged (using a counter-balance) for 5 min at a setting just above zero to pellet the cells. The freezing medium was carefully vacuumed off so as not to have disturbed the cell pellet. The cells were re-suspended in 5 mL of complete media and the suspension was transferred to a clean flask. The lid was left loose so as to allow air to flow to the cells. The flask was carefully placed in the incubator so as not to let media splash up into the neck of the flask.

Cells that have been brought up from the freezer are generally ready to split the following day. The splitting process began with the flaming of both the neck of the flask and the tip of the vacuum. The hood was sterilized with ethanol and a new glass vacuum

pipette tip was connected to the vacuum line. The complete media and the trypsin were warmed in a 37 °C water bath. When the cells reached confluency (when they appeared under microscopic magnification to be visibly clumped together), they were ready to be split.

The cells were split 1:5 (a single confluent flask was divided into five new ones). The vacuum tip and the neck of the flask were flamed before old media was suctioned off. Care was taken not to touch the sides of the flask or the cells with the tip. The cells were washed with 5 mL 1X sterile HEPES. Next, 1 mL 1X trypsin was added. The flask was rested on a flat surface in order to enable the trypsin to cover all of the cells. The cells were viewed under microscopic power, and watched while they rounded up and detached. The flask was tapped gently until the cells dislodged from the bottom of the flask. Then 4 mL of complete media was then added (the total volume in the flask will be 5 mL since 1 mL of trypsin is still in the flask). Using a pipette, the contents were drawn up and down to dislodge the cells from the side of flask and to mix the contents thoroughly. Next, the 5 mL volume was transferred into 5 separate flasks (1 mL per flask). Then 4 mL of complete media was added to each flask for a final volume of 5 mL per flask. The lids were affixed loosely so that air was able to flow to the cells. The flasks were carefully placed in the incubator, being careful so as not to let media splash up into the neck of the flask. The cells were ready to be split again a few days later.

"Day One" of the experiment constituted "cell seeding." The hood was sterilized as described before, and, additionally, the UV lamp was turned on for about 15 minutes. The cells were counted and split so that approximately 10,000 cells/200 μL were added to

each well of the plate. The protocol for splitting cells was then followed. A small sample (~20 μL) of cells was obtained. The following formula was used to count them accurately: (Cells counted grid 1 + cells counted grid 2+ cells counted grid 3 + cells counted grid 4)/4. This number was multiplied by 10,000, then multiplied again by the number of milliliters that the cells were re-suspended in (in this example, 5 mL). This provided the total number of cells within the 5 mL volume. This volume was divided by 5 to obtain the number of cells/mL. This new value was then divided by 5 again to obtain the number of cells/200 mL. The cells were diluted accordingly to obtain approximately 5,000 cells/200 μL well. After counting, the cells were spun down and resuspended in enough folate-free medium to give an appropriate number of cells per well. The 50 mL Falcon tube was used to do this. The contents of the tube were mixed thoroughly by pipetting up and down (alternatively, one could have inverted the tube). Next, 200 μL of the cell mixture was added to each well. It was not necessary to change the pipette tip, but extreme care was taken so as not to contaminate the cells. The cells were ready for drug exposure after 24 hr.

"Day Two" of the experiment constituted "Drug Exposure." The hood was sterilized with the UV lamp turned on, as before. The folate free media was removed by vacuum, and then the drug was added. Additional folate free media was added as needed to achieve a final volume of 200 μL. The well-plates were placed inside of a box, and then the box was placed inside of the incubator for 24 hr.

"Day 3" of the experiment constituted "Light Exposure." The hood was sterilized with the UV lamp turned on, as before. The media was removed by vacuum and the cells

were washed twice with HEPES. Then 200 μL of complete (folate containing) media was added, and the cells were placed under the light source. For the well-plate designated as the "light plate," the foil-wrapped cardboard shutter was repositioned in order to permit light exposure to a given row of cells at 1 minute, 2 minute, 4 minute, and 6 minute intervals. The two plates were then placed in the box, and the box was then placed inside of the incubator for 24 hr.

"Day 4" of the experiment constituted "Cell-Titer-Blue Death Assay." The hood was sterilized with the UV lamp turned on, as before. The media was removed by vacuum and the cells were washed twice with complete (folate containing) media. Then100 μL of complete media was then added to the cells. A blank column was set up containing only the media (no cells). Next, 20 μL of the cell-titer-blue death assay was added to each well, including the blank wells (this was done for both plates). The two plates were placed in the box, and then the box was placed inside of the incubator for 2 hr. Afterwards, both plates were taken to Dr. Williamson's lab (Biology Department) and analyzed at 590 nm on the plate reader.

An image of a 96-well plate reflecting assignments for an experiment run to develop pipetting technique (that could also serve to provide preliminary results) is shown below:

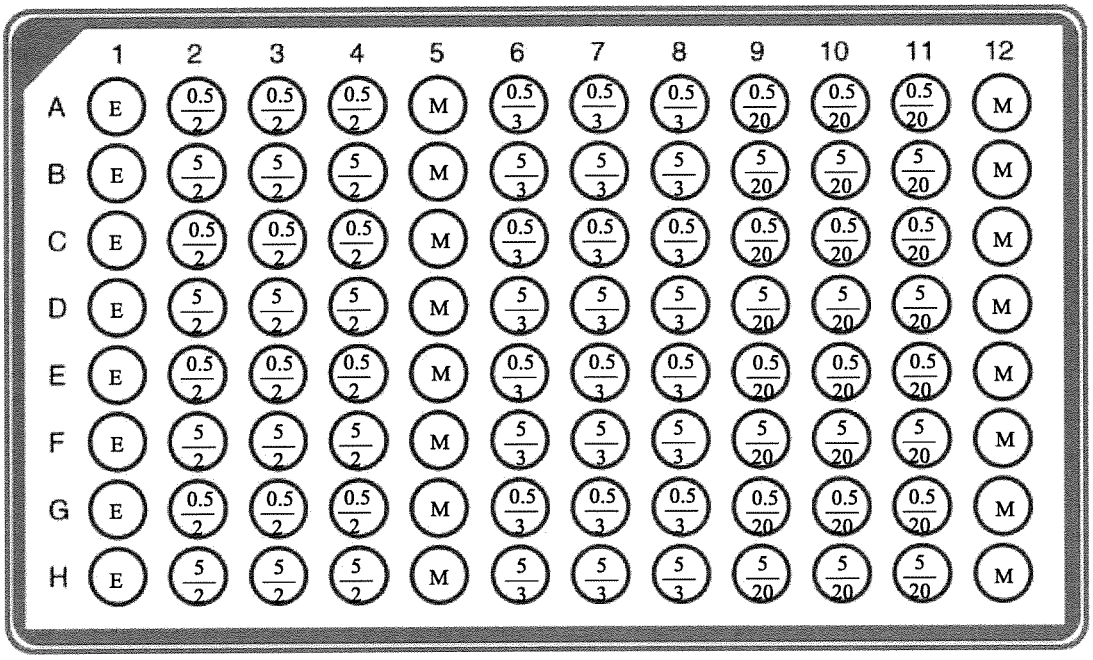


Figure 1. 96-well plate assignments for an experiment to develop pipetting technique.

Each well is designated as follows:

E → empty (no cells, no media, no PDT drug) **M** → media with cells, no PDT drug

5/2 → 5 μM solution of PEG conj 2000 **0.5/2** → 0.5 μM solution of PEG conj 2000

5/3 → 5 μM solution of PEG conj 3400 **0.5/3** → 0.5 μM solution of PEG conj 3400

5/20 → 5 μM solution of PEG conj 20000 **0.5/20** → 0.5 μM soln of PEG conj 20000

Both plates (the one designated as dark and the other designated as light) received the same well assignments. For the plate being exposed to light, rows A – B received 1 minute light exposure; rows C – D received 2 minutes; rows E – F received 4 minutes; rows G – H received 6 minutes.

The first task for the practice run was to make up a 40 μM stock solution of the 2,000 conjugate. It was intended that 1 mg of 2,000 conjugate would be diluted in a

volumetric flask with 9.69 mL PBS (from the previously listed table). L. Donahue's ampule of 2000 conjugate from the freezer; however, was labeled as containing only 100 μL of solution, originally coming from a 0.1 mg/mL stock solution. It was determined that the amount of 2000 conjugate in the ampule needed to be calculated:

$$\frac{100\ \mu L\ PEG_{2000}\ conjugate \mid 1\ mL \mid 0.1\ mg}{1000\ \mu L \mid 1\ mL} = 0.01\ mg$$

The contents of the ampule were transferred into a 5 mL volumetric flask and stripped off the methanol with a continual stream of nitrogen gas. The volume of PBS to be added to the volumetric flask was then calculated:

$$\frac{0.01\ mg\ PEG_{2000}\ conjugate \mid 9.69\ mL \mid 1000\ \mu L}{1\ mg \mid 1\ mL} = 96.9\ \mu L\ PBS$$

This experiment was designed with the intention of each well containing 75 μL of stock solution (whether it be 2,000 conjugate, 3,400 conjugate, or 20,000 conjugate). This way, the experimentalist could take 25 μL for the light plate, another 25 μL for the dark plate, and have 25 μL left over to minimize pipet error. The design of the experiment was for 12 wells to contain 40 μM stock solution, and for another 12 wells to contain 4 μM stock solution, that way, when one adds 175 μL of media to the 25 μL portions of stock solution already measured into each well, the total volume in each well becomes 200 μL. Calculating the new concentration: 25 μL of 40 μM solution/200 μL media = 0.125; and, (40 μM)(0.125) = 5 μM. Table 5 (previously reported) can again be referred

to for determining specified concentrations of drug that the cells could potentially be introduced to for future assays:

It was soon realized that 97 μL of total stock solution was not going to be nearly enough to fill multiple wells at a volume of 75 μL-per-well. Furthermore, this experiment was designed with the intention of measuring drug effectiveness at both 5 μM concentration and at 0.5 μM concentration. Thus, some of this 97 μL stock solution would need to be allocated towards making a 4 μM stock solution. At this point, the thought of filling each well with less than 75 μL was considered. It was estimated that 5 μL-per-well would be a small enough quantity to stretch the available compound. The following calculations were performed: (12 wells)(5 μL per well) = 60 μL of 40 μM stock solution required for the cell-kill study; and, 97 μL – 60 μL = 37 μL stock solution remaining for making up the 4 μM stock solution. Note: 4 μM is 10 times as dilute as 40 μM. Next, 30 μL of the remaining 37 μL of 40 μM solution was transferred to a clean test tube. The total volume of the 4 μM stock solution was calculated, starting from 30 μL of available 40 μM solution: (30 μL)(10) = 300 μL solution. The available 30 μL was diluted with 270 μL PBS to bring the contents of the test tube to a total volume of 300 μL: (12 wells)(5 μL per well) = 60 μL of 4 μM stock solution now required for the cell-kill study. Although having 240 μL of the 4 μM left over makes it look as if one could have filled each well with considerably more than 5 μL of solution, recall that one would not have been able to do the same for the 40 μM solution.

The strategy was then to maintain consistency between well-volumes. Each well was filled with 5 μL, affording 2.5 μL for the light plate, another 2.5 μL for the dark

plate, and 0 μL left over for minimizing pipet error. A visual check of the following was performed: (1) the pipet setting remained on 5 μL, (2) the outside of the pipet tip did not have residual droplets of solution, and (3) the inside of the pipet tip did not have air bubbles, and there appeared to be a consistent amount of liquid in the tip, from one filling to the next.

For preparation of future 2000 conjugate 40 μM stock solutions, the following is recommended: (1) synthesize and purify enough 2000 conjugate to be able to measure out 2.0 mg of material in a 25 mL volumetric flask, and (2) add 19 mL + 380 μL PBS.

The next task for the practice run was to make up a 40 μM stock solution of the 3,400 conjugate. It was intended that 1 mg of 3,400 conjugate was to be diluted in a volumetric flask with 6.25 mL PBS. In practice, 1.3 mg of 3400 was actually measured into a 25 mL volumetric flask. It was then calculated that (6.25 mL PBS)(1.3) = 8.13 mL PBS would be required. A pipet was used to add 8 mL + 130 μL for accuracy.

Similarly, a 40 μM stock solution of the 20,000 conjugate was to be prepared. It was intended that 1 mg of 20,000 conjugate was to be diluted in a volumetric flask with 1.21 mL PBS. In practice, 4.5 mg of 20,000 was actually measured into a 10 mL volumetric flask. It was then calculated that (1.21 mL PBS)(4.5) = 5.45 mL PBS would be required. A pipet was used to add 5 mL + 450 μL for accuracy.

The 4 μM stock solution for the 2,000 conjugate was already prepared (as previously discussed); however, preparation of equivalent 4 μM stock solutions for the 3,400 conjugate and the 20,000 conjugate were performed as follows: 30 μL of the

respective 40 μM stock solutions were transferred into clean test tubes, followed by addition of 270 μL PBS to each tube.

The data collected from the assay revealed that control columns (5 and 12) did not produce the expected high fluorescence count. This suggests that the data might be invalid. Furthermore, CO_2 levels might not have been homogeneous throughout the exposure chamber.

The remaining Cell-Kill experiments were planned for which conjugate 2000 was to be substituted with chlorin-e6. An image of a 96-well plate reflecting assignments for each of these experiments is shown below:

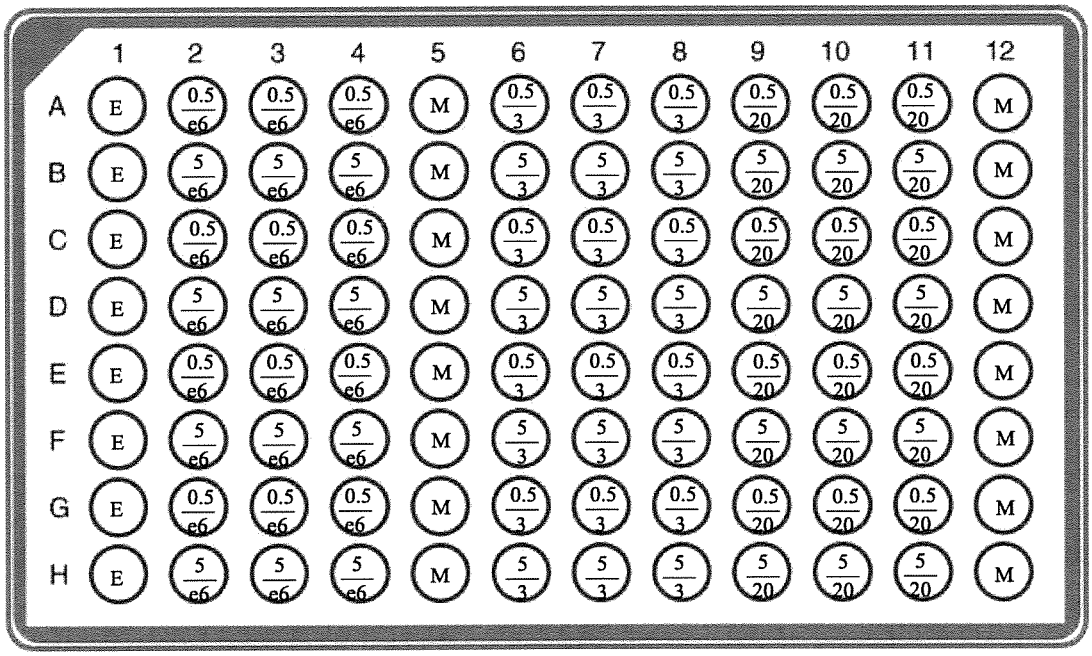


Figure 2. 96-well plate assignments for all experiments that compare e6 with conjugates. Each well is designated as follows:

E → empty (no cells, no media, no PDT drug) **M** → media with cells, no PDT drug

5/e6 → 5 μM solution of Chlorin e6 **0.5/e6** → 0.5 μM solution of Chlorin e6

5/3 → 5 μM solution of PEG conj 3400 **0.5/3** → 0.5 μM solution of PEG conj 3,400

5/20 → 5 μM solution of PEG conj 20,000 **0.5/20** → 0.5 μM soln of PEG conj 20,000

Both plates (the one designated as dark and the other designated as light) received the same well assignments. Each well contained 75 μL of drug/PBS solution (25 for light, 25 for dark, 25 extra). For the plate being exposed to light, rows A – B received 1 minute light exposure; rows C – D received 2 minutes; rows E – F 4 received minutes; rows G – H received 6 minutes.

A 40 μM stock solution of chlorin e6 was then prepared. It was intended that 1 mg of chlorin e6 was to be diluted in a volumetric flask with 41.88 mL PBS. In practice, 2.0 mg of chlorin e6 was measured into a 125 mL erlenmeyer flask. It was then calculated that (41.88 mL PBS)(2.0) = 83.76 mL PBS would be required. A 25 mL volumetric pipet was used to add 75 mL (3 x 25 mL) PBS, then a blue-tip pipet was used to add 8 ml and again used to add 760 μL for accuracy. Preparation of a 4 μM stock solution of chlorin e6 was prepared by transferring 300 μL of the 40 μM stock solution into a clean test tube and then adding 2700 μL PBS to the test tube.

The cells were seeded and given 24 hr to mature. The drug was then introduced, and the cells were given 24 hr exposure. Exposure to near-IR light (lamp suspended 3 inches above the light plate) at previously discussed durations was conducted, and again, the cells were given 24 hours to react. Finally, assays were conducted on both plates.

The following bar graphs were generated as an average of three separate, but equivalent, cell-kill studies (assays obtained on August 12, 2015, August 24, 2015, and

September 1, 2015). Standard error bars have been included. Overlap of these bars implies non-significant variance:

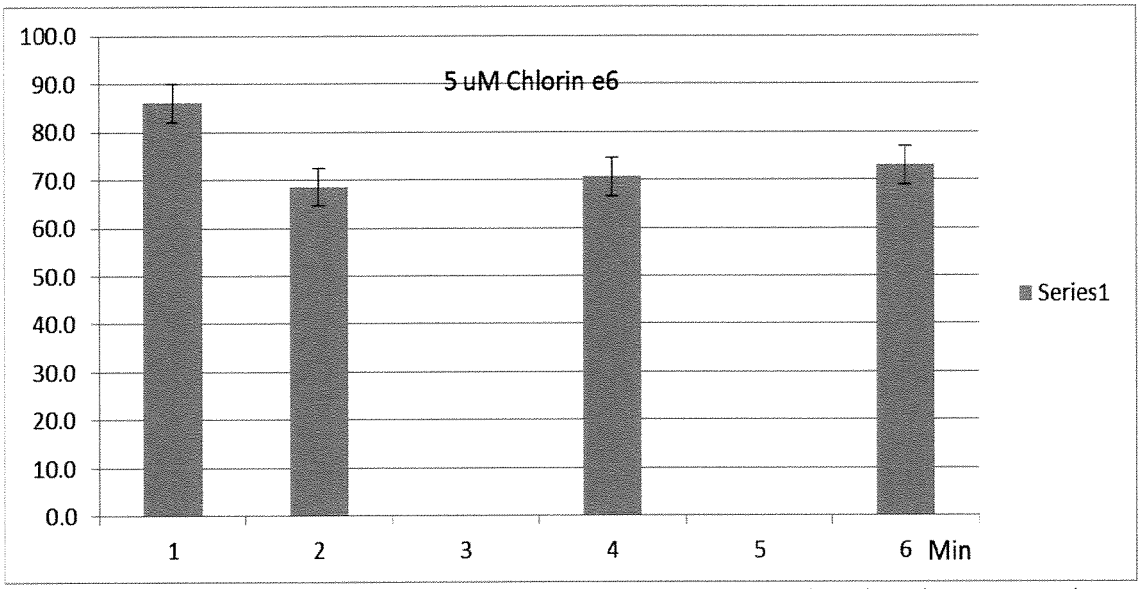


Figure 3. Chlorin e6 at 5 μM concentration (x-axis represents time in minutes; y-axis represents fluorescence count in relative percentage).

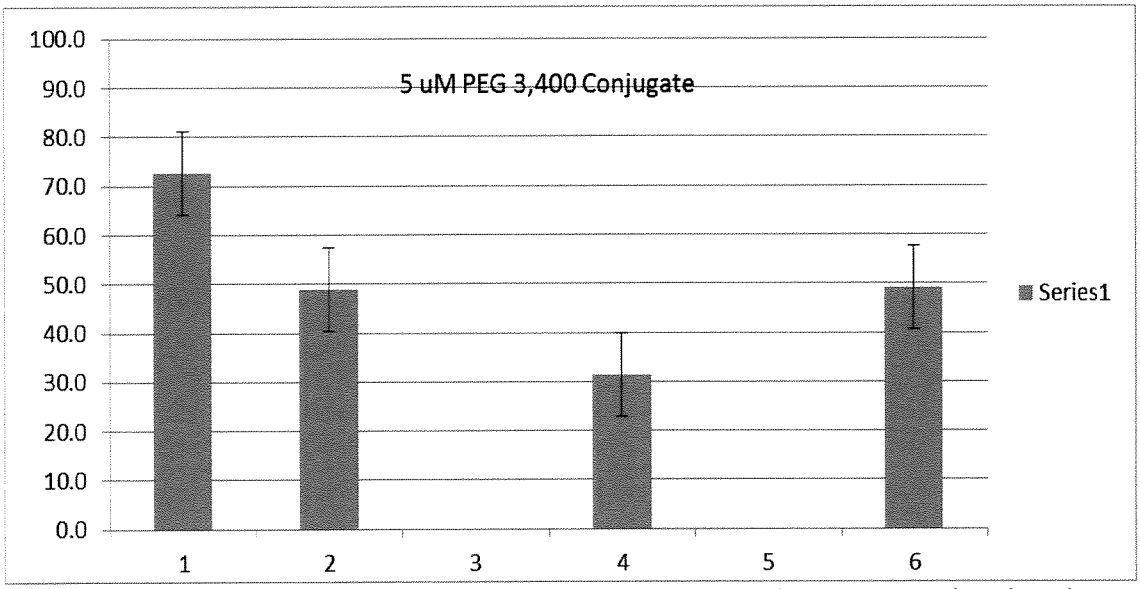


Figure 4. PEG 3,400 Conjugate at 5 μM concentration (x-axis represents time in minutes; y-axis represents fluorescence count in relative percentage).

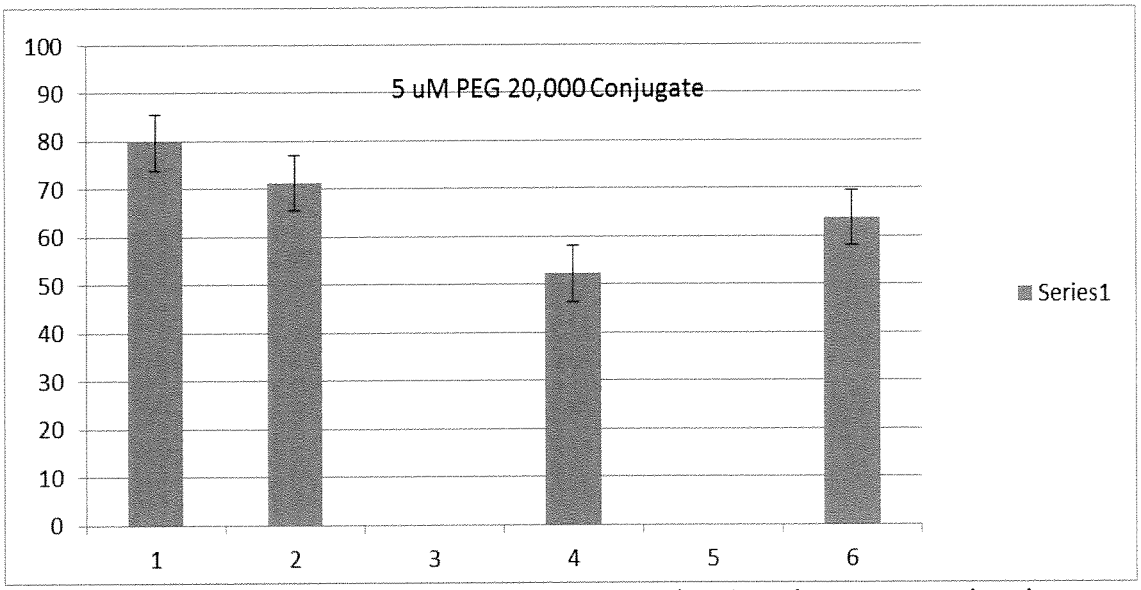


Figure 5. PEG 20,000 Conjugate at 5 μM concentration (x-axis represents time in minutes; y-axis represents fluorescence count in relative percentage).

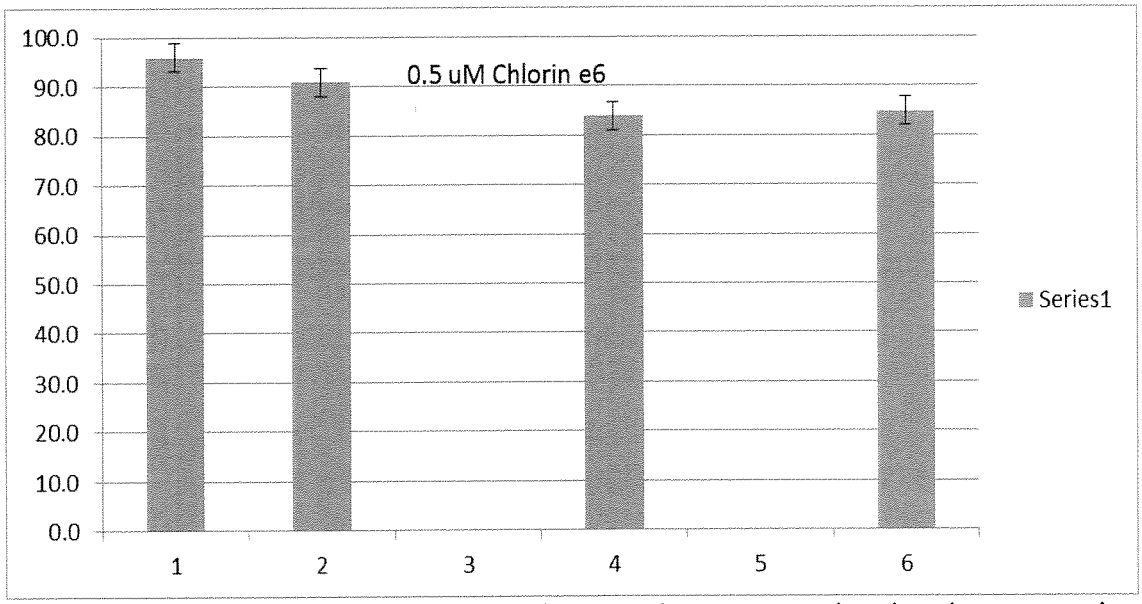


Figure 6. Chlorin e6 at 0.5 μM concentration (x-axis represents time in minutes; y-axis represents fluorescence count in relative percentage).

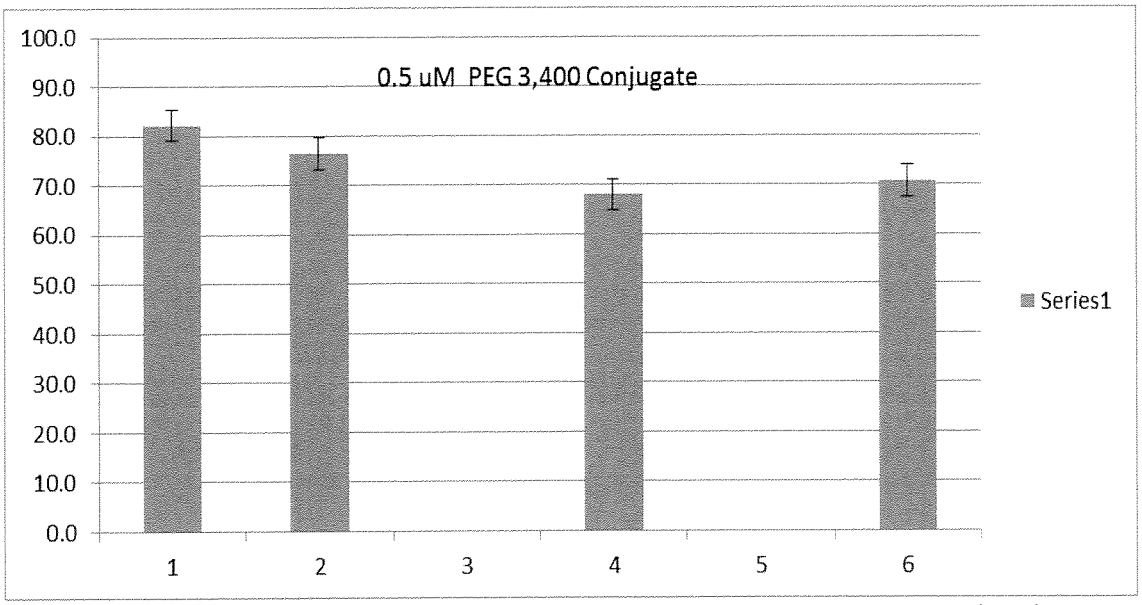


Figure 7. PEG 3,400 Conjugate at 0.5 μM concentration (x-axis represents time in minutes; y-axis represents fluorescence count in relative percentage).

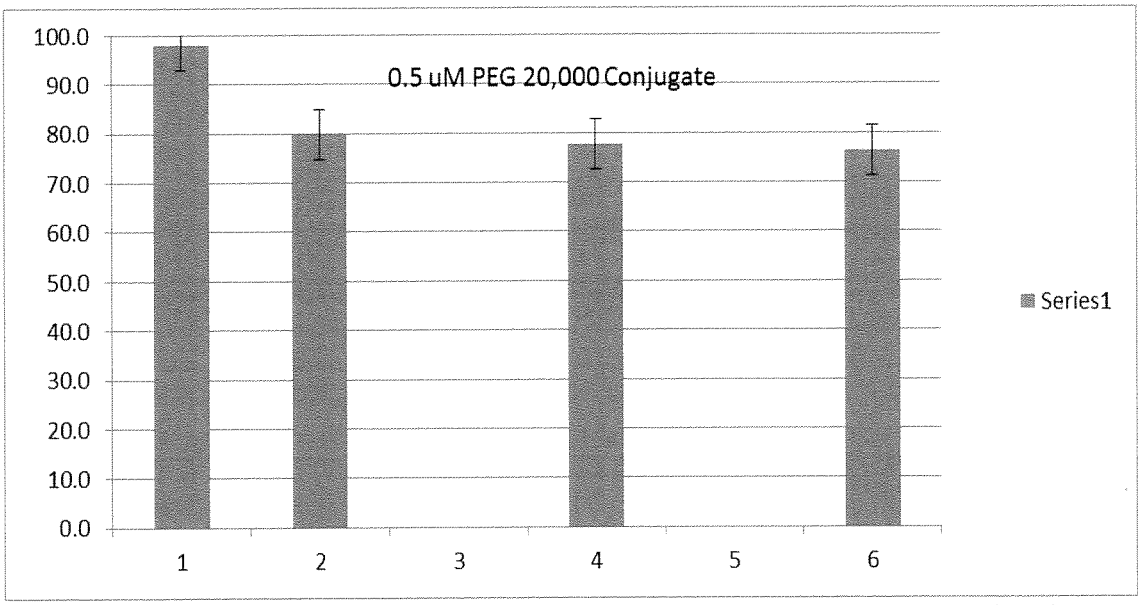


Figure 8. PEG 20,000 Conjugate at 0.5 μM concentration (x-axis represents time in minutes; y-axis represents fluorescence count in relative percentage).

From the bar graphs that represent the per cent of the cells that survived the cell killing studies, it can be concluded that chlorin e6 is only moderately more effective at 5 μM than at 0.5 μM (analogous to L. Donahue's Chlorin e6 findings for 10 μM versus 5 μM,

suggesting that even 5 μM may be more concentrated than necessary). It appears that for both concentrations of chlorin e6 being compared, prolonged exposure to light does not decrease fluorescence count significantly. Similar analysis of the 3,400 PEG conjugate at 5 μM and 0.5 μM concentrations reveals that increased concentration does reduce fluorescence count significantly, particularly after 4 min of light exposure. Furthermore, analysis of the 20,000 PEG conjugate at 5 μM and 0.5 μM concentrations reveals that increased concentration does reduce fluorescence count significantly, however, this is not until after 4 minutes of light exposure. Finally, comparisons between chlorin e6, 3,400 PEG conjugate, and 20,000 PEG conjugate reveals that PEG conjugates are more effective at killing cells than chlorin e6, with increasing length of PEG amine being inversely proportional to cell-killing effectiveness. Additional studies should be conducted to compare the cell killing efficiencies of the 2,000 and 10,000 PEG conjugates at both 5 μM and 0.5 μM to the results reported here.

CHAPTER THREE

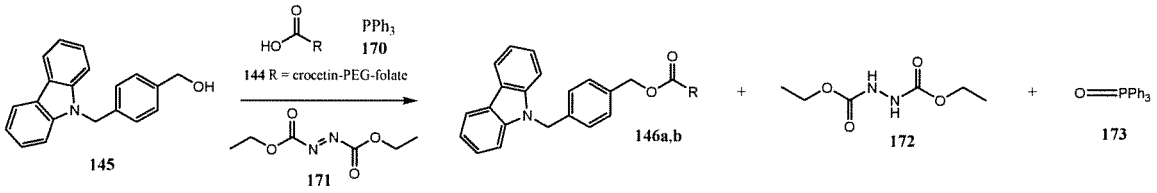
FUTURE STUDIES

The first priority for future study would be to repeat in-vitro cell-kill studies at nanomolar concentrations on the Chlorin e6-PEG-amine-folate conjugates. If positive cell-kill results, assays can be repeated at picomolar concentrations, and possibly femptomolar concentrations. If negative cell-kill results, assays can be repeated with increased duration of exposure to near-IR light. Compilation of this data would then be used in order to establish a measure of drug potency for PDT.

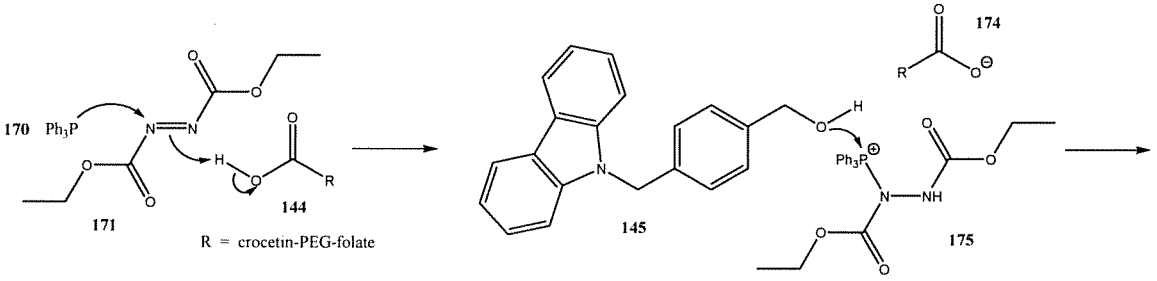
The next priority would be to continue pursuing the installation of crocetin as a UV-spacer. Carbazole-protected benzyl linker **145** (and its dibrominated analog) would be attached to one end of crocetin, and PEG-amine-folates (specifically the ones which provided the highest cell-kill data when coupled with chlorin e6) would be attached to the other end. These targets **146a,b** would enable determination of whether additional conjugation and halide dipole moment extends UV absorption beyond that observed in **144**, and if so, whether it is a negligible difference (661 – 699 nm), a significant increase (700 – 899 nm), or too great of an increase (>899 nm). These targets would also enable determination of whether or not two photosensitizers (crocetin and carbazole) within the same molecule would express a combined effect towards in-vitro cell death. If the carbazole-crocetin-PEG-amine-folate conjugate at previously measured concentrations do not show a greater decrease in fluorescence count, a comparison of the new count with

those of independent crocetin conjugates and independent carbazole conjugates could reveal whether one photosensitizer was dominant.

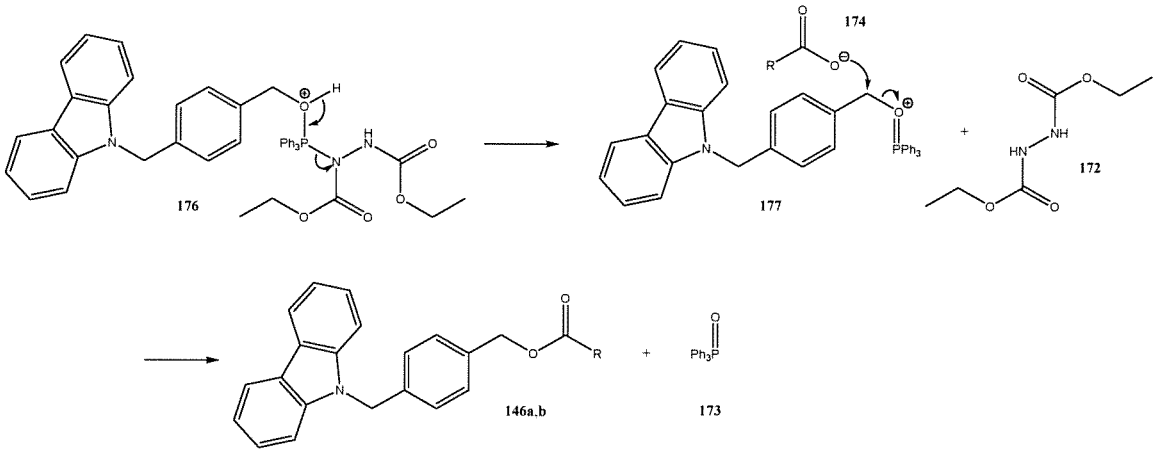
Preparation of **146a,b** would initially be attempted using Fisher esterification conditions, with DCC and possibly HOBT as assisting reagents. Alternatively, Mitsunobu conditions, diethylazodicarboxylate (DEAD) **171** and PPh_3, could be employed.[68]



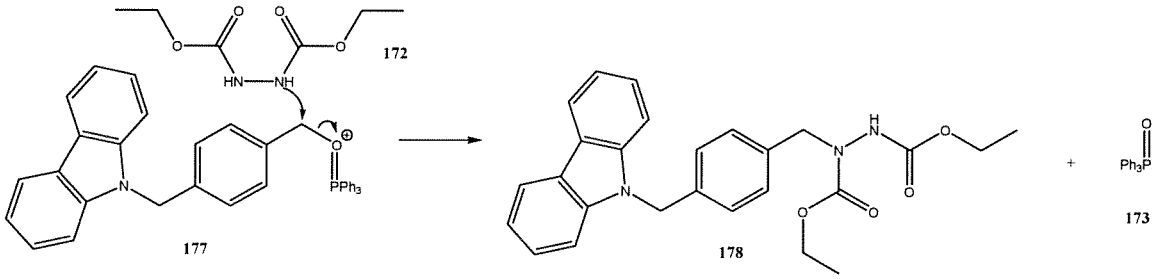
The principle behind the Mitsunobu is that triphenylphosphine functions as a nucleophile, attacking the azo functionality of **171**. That azo nitrogen picks up a proton from crocetin, generating a carboxylate anion.



Elimination of diethylhydrazidedicarboxylate **172** generates a triphenylphosphine oxide leaving group as part of **177**, which is subsequently displaced by the crocetin carboxylate **174** formed *in situ*, resulting in ester formation.



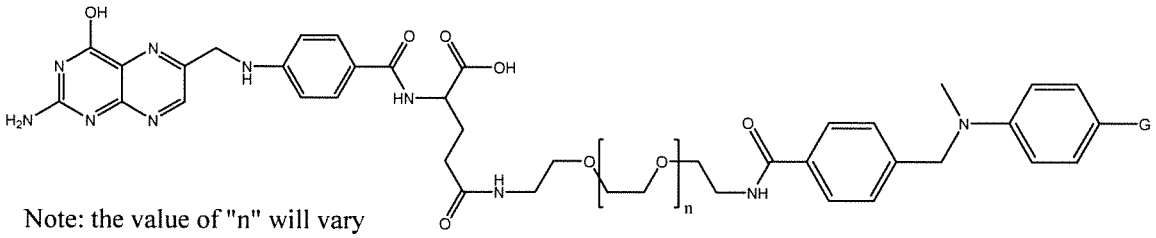
The advantage of the Mitsunobu over the Fisher would be that the Mitsunobu would not introduce strongly acidic catalysts that might lead to unwanted isomerizations within crocetin's diterpene chain. The disadvantage of the Mitsunobu, however, would be that it might lead to an additional (unwanted) final product **178** resulting from nucleophilic diethylhydrazidedicarboxylate by-product/intermediate **172** forming *in situ* and competitively displacing triphenylphosphine oxide **173**.



It is worth noting that the Mitsunobu is known to invert chirality at the alcohol position by an S_N2 mechanism. The ester oxygen in **146a,b** occupies an achiral benzylic position, however future analogs might employ a stereogenic center at this position.

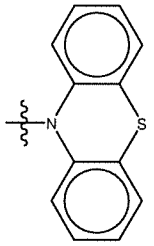
Thirdly, the investigators would revisit **83**, the *N*-methyl-4-bromoaniline sensitizer, preparing analogs (via Buchwald-Hartwig) **179-183** which vary in the extent to

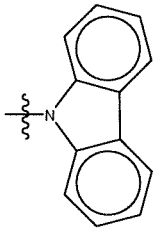
which their para-substituent serves to activate or deactivate the phenyl linker. Conjugates would be prepared in accordance with 4-bromobenzylcarbomethoxy chemistry and PEG-folate chemistry previously discussed.



Note: the value of "n" will vary

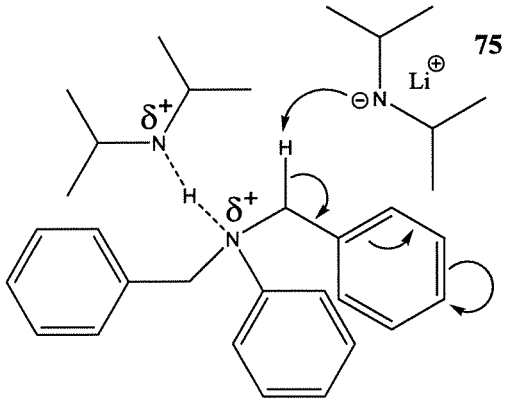
G = H, Br, NO_2,





179 **180** **181** **182** **183**

Finally, studies on benzyl deprotection would be resumed. In the proposed base-catalyzed methodology of benzyl amine deprotection, it is suspected that initial deprotonation of the benzylic position was not occurring, due to insufficient basicity of lithium diisopropyl amide. It would be prudent to replace LDA with the more strongly basic but equivalently-hindered tert-butyllithium reagent. Alternatively, LDA could be diluted with additional diisopropyl amine, in an effort to promote hydrogen bonding with the benzyl nitrogen through polar-protic solvation. Dilution would reduce collision frequency between LDA and **60**, extending the overall time required for deprotonation to occur. However, solvation might increase the benzylic nitrogen's positive character, which in turn might decrease the neighboring benzyl proton's pK_a enough for LDA to remove it in small equilibrium.

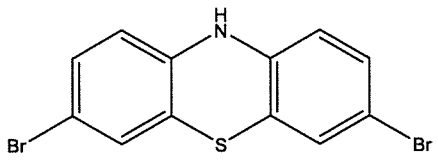


60

Other methodologies for benzyl amine deprotection, such as high pressure catalytic hydrogenation, are known, but feature harsh conditions that lack chemoselectivity (particularly with regards to aliphatic olefins, such as those found within the diterpene chain of crocetin).[69] This proposed base-catalyzed methodology, however, if successful (on **60** and on a small library of other selected benzyl amine systems), would suggest publication of a novel, facile route for selective benzyl amine deprotection by a mechanism that has not yet been reported.

CHAPTER FOUR

EXPERIMENTAL

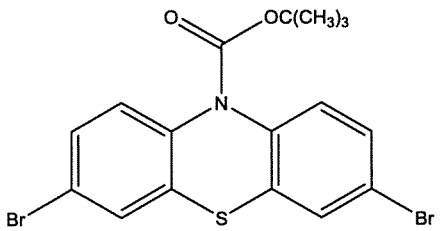


16

3,7-dibromo-10*H*-phenothiazine (16). CAS 21667-32-3

This compound has been previously reported by Ikeda *et al.*[70] The method for which its preparation is based upon, has been previously reported by Sang *et al.*[71] To an oven-dried 250 mL round bottom flask containing phenothiazine (**8**) (1 g, 5.03 mmol) was added CCl_4 (30 mL), and the suspension was heated to 65°C and stirred until all material had dissolved. The resulting solution was allowed to gradually cool to 30°C and to it was added a solution of bromine (ACS grade) (0.24 mL) in CCl_4 (5 mL) at a rate of 5 drops per second. After stirring the reaction at 30°C for 30 min, hot (not boiling) toluene (100 mL) was added, resulting in a black slurry. Sodium dithionite (tech grade) (6 spatulas) was added to quench the reaction, and the slurry was immediately vacuum filtered. The filtrate was concentrated under vacuum to afford dark green crystals (0.496 g, 30% yield). The black precipitate was transferred to another batch of hot toluene (100 mL), vacuum filtered, and the filtrate was concentrated under vacuum and pumped to afford light green crystals (0.577 g, 35% yield). Purification, if necessary, could be brought

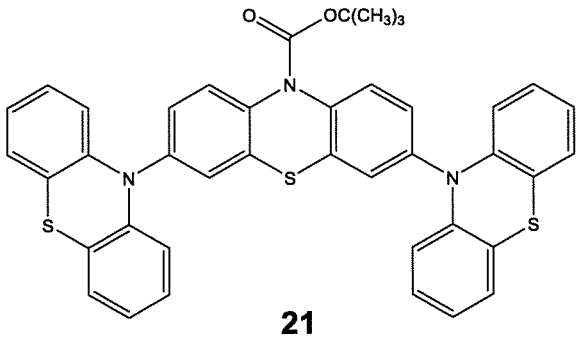
about by dissolving separate batches of crystals in acetonitrile (200 mL) at room temperature with ultrasonic bombardment (5 min), then allowing the mother liquor to sit for 12 or more hours in the dark. Upon crystal formation, the mother liquor could be decanted and the crystals allowed to dry: mp 100 °C; TLC R_f 0.69 (1% EtOAc/pet ether).



20

***Tert*-butyl 3,7-dibromo-10*H*-phenothiazine-10-carboxylate (20). CAS 781660-75-1**

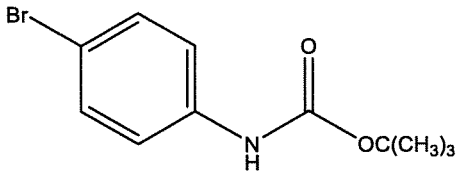
This compound has been previously reported by Clarke *et al.*[72] The method for which its preparation is based upon has been previously reported by Basel *et al.*[73] A mixture of 3,7-dibromo-10*H*-phenothiazine (**16**) (100 mg, 0.28 mmol), di-*tert*-butyl dicarbonate (**19**) (73 mg, 0.33 mmol), 4-dimethylaminopyridine (3.5 mg, 0.032 mmol) as a proton scavenger, and anhydrous acetonitrile (freshly distilled from CaH_2) (10 mL) was refluxed under a nitrogen atmosphere for 24 h. The crude product was concentrated under vaccum, dissolved in CH_2Cl_2, washed with de-ionized water to remove aqueous-soluble 4-dimethylaminopyridine (2 x 10 mL), and again concentrated under vacuum and pumped to afford **20** as a green solid. The crude product was dissolved in minimal toluene and gravity chromatographed (silica gel pre-soaked in NH_4OH to prevent carbamate deprotection, eluent 1% EtOAc/pet ether): TLC R_f 0.69 (1% EtOAc/pet ether).



21

***Tert*-butyl 10'*H*-[10,3':7',10"-terphenothiazine]-10'carboxylate (21)**

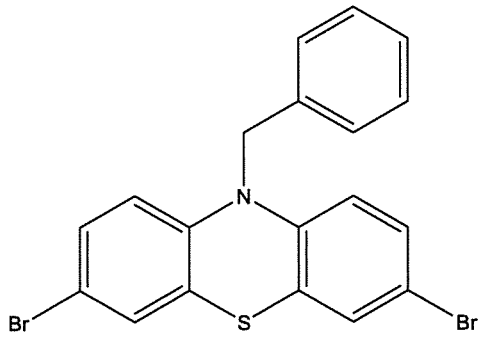
This compound, as well as the method for which its preparation is based upon, has been previously reported by Sanjeevaiah.[74] To a 25 mL 3-neck round bottom flask containing a solution of tri-t-butylphosphine in o-xylene (0.5 mL, 0.1 M) and anhydrous toluene (1.2 mL) was added *tert*-butyl 3,7-dibromo-10*H*-phenothiazine-10-carboxylate (**20**) (100 mg, 0.22 mmol), phenothiazine (**8**) (88 mg, 0.44 mmol), bis(dibenzylideneacetone) palladium(0) (5 mg, 0.009 mmol), and sodium t-butoxide (73 mg, 0.759 mmol). The reaction was refluxed for 90 min under an argon atmosphere. Hot filtration through a fritted funnel was performed to remove solid impurities, and the filtrate was concentrated under vacuum. The crude product was dissolved in minimal toluene and gravity chromatographed (silica gel, 1% EtOAc/pet ether) to afford **21**: TLC R_f 0.69 (1% EtOAc/pet ether).



25

***Tert*-butyl (4-bromophenyl) carbamate (25). CAS 131818-17-2**

This compound has been previously reported by Wasley *et al.*[75] The method for which its preparation is based upon has been previously reported by Basel *et al.*[73] To a 100 mL round bottom flask containing a spin bar and p-bromoaniline (**24**) (0.78 g, 4.55 mmol) was added di-*tert*-butyl dicarbonate (**19**) (1.98 g, 9.09 mmol), 4-dimethylaminopyridine (27.3 mg, 0.248 mmol), and anhydrous acetonitrile (22.5 mL). The reaction was refluxed under a nitrogen atmosphere for 24 h. The light gold-tinted suspension was concentrated under vacuum, combined with de-ionized water (20 mL), and decanted into a separatory funnel. The water layer was extracted with dichloromethane (2 x 20 mL) and the combined organics were concentrated under vacuum to afford 45.5% conversion (as determined by proton NMR) to **25** as a gold oil, which formed gold crystals while air drying: TLC R_f 0.85 (40% EtOAc/pet ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (d, 2H), 7.16 (d, 2H), 6.47 (bs, 1H), 1.52 (s, 9H).



39

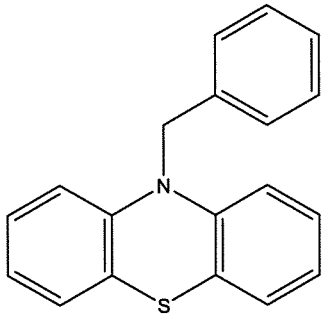
10-benzyl-3,7-dibromo-10*H*-phenothiazine (39) (*Prep #1*)

This compound does not appear to have been previously reported. The method for which its preparation is based upon has been previously reported by Bordwell *et al.*[76] To an oven-dried 50 mL round bottom flask containing a spin bar was added potassium hydride slurry (30% KH/mineral spirits) (97 mg slurry, 0.728 mmol actual reagent). The mineral spirits were removed with petroleum ether washes (3 x 3 mL freshly distilled from CaH_2), and to the potassium hydride was immediately added *N*,*N*-dimethylformamide (DMF) (1 mL), followed by a solution of 3,7-dibromo-10*H*-phenothiazine (**16**) (285 mg, 0.798 mmol) in DMF (1.65 mL). The reaction was stirred under nitrogen for 10 minutes. To the reaction was then added benzyl bromide (**43**) (682 mg, 3.99 mmol) dropwise, and the reaction was stirred for 15 minutes. The reaction was quenched with de-ionized water (5 mL), stirred for 20 minutes, extracted with CH_2Cl_2 (3x15 mL), washed with de-ionized water, and washed with brine. The combined organics were treated with sodium dithionite, filtered through fritted glass, and concentrated under vacuum. The brownish-green oil was dissolved in minimal CH_2Cl_2 for purification by chromatography (silica, 100% pet ether), TLC R_f 0.56 (5% Et2O/pet ether) afforded 83.5 mg (23.4%) of **39** as a

light green solid. mp 194-197 °C, ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (m, 5H), δ 7.23 (d, 2H), δ 7.07 (d, 2H), δ 6.46 (d, 2H), δ 5.01 (s, 2H).

10-benzyl-3,7-dibromo-10*H*-phenothiazine (39) (*Prep #2*)

This compound does not appear to have been previously reported. The method for which its preparation is based upon has been previously reported by Sang *et al.*[71] To a 100 mL round bottom flask containing a spin bar and 10-benzyl-10*H*-phenothiazine (**40**) was added CH_2Cl_2 (13 mL), followed by bromine (0.75 mL, 14.5 mmol) and a CH_2Cl_2 rinse (1.6 mL). The reaction was stirred at room temperature for 4 hours. The reaction was quenched with aqueous NaOH (15 mL dropwise addition, 1.0 M), extracted with CH_2Cl_2 (3 x 15 mL), and washed with brine (3 x 15 mL). The combined organics were dried with sodium dithionite, filtered through fritted glass, and concentrated under vacuum to afford **39** as a bluish-green oil that could be purified by the same methods as reported in Prep #1.

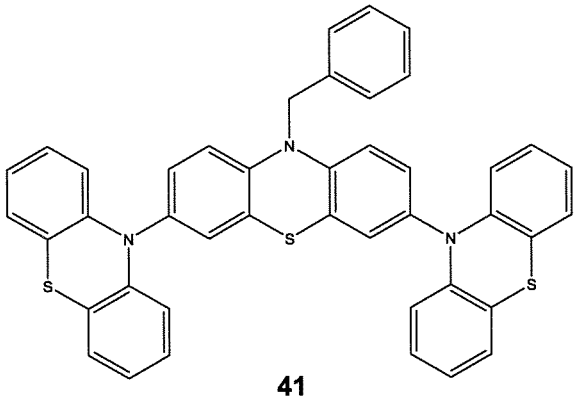


40

10-benzyl-10*H*-phenothiazine (40). CAS 58478-75-4

This compound, as well as the method for which its preparation is based upon, has been previously reported by Bordwell *et al.*[76] To an oven-dried 15 mL 3-neck round bottom

flask containing a spin bar was added potassium hydride slurry (30% KH/mineral spirits) (168 mg slurry, 1.26 mmol actual reagent). The mineral spirits were removed with hexane washes (3 x 3 mL), and to the potassium hydride was immediately added *N*,*N*-dimethylformamide (DMF) (0.5 mL). A solution of 10*H*-phenothiazine (**8**) (279 mg, 1.40 mmol) in DMF (2.3 mL) was added, followed immediately afterwards by streamwise addition of benzyl bromide (**43**) (0.3 mL, 3.99 mmol). The reaction was stirred for 48 hours. The reaction was quenched with de-ionized water, stirred for 60 minutes, extracted with CH_2Cl_2 (2 x 15 mL), washed with de-ionized water, and washed with brine. The combined organics were dried with sodium dithionite, filtered through fritted glass, and concentrated under vacuum to afford 292 mg (73.7%) of **40** as a greenish-brown oil: TLC R_f 0.47 (10% Et2O/pet ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31 (m, 5H), δ7.07 (d, 2H), δ 7.00 (m, 2H), δ 6.89 (m, 2H), δ 6.63 (d, 2H), δ 5.09.

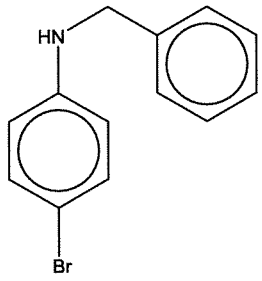


41

10'-benzyl-10'*H*-10,3':7',10"-terphenothiazine (41)

This compound does not appear to have been previously reported. The method for which its preparation is based upon has been previously reported by Ferreira *et al.*[77] To a 75 mL

pressure flask containing a spin bar and 10-benzyl-3,7-dibromo-10*H*-phenothiazine (**39**) (450 mg, 1.01 mmol) was added 10*H*-phenothiazine (**8**) (802 mg, 4.04 mmol), tris(dibenzylideneacetone)dipalladium(0) (52 mg, 0.051 mmol), 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (BINAP) (47 mg, 0.076 mmol), cesium carbonate (1.32 g, 4.04 mmol), and toluene (9 mL). The flask was purged with argon, sealed, and refluxed for 120 hours between 140-160 °C. The reaction was gravity filtered, chased with CH_2Cl_2, and the filtrate concentrated under vacuum to afford brown crystals. Purification by chromatography (silica, 10% Et_2O/pet ether) removed unreacted phenothiazine: TLC (10% Et2O/pet ether) R_f 0.10. Remaining UV active compounds include R_f 0.46, 0.43, 0.38, 0.31, 0.26, 0.16, and 0.13, with 0.26 thought to be the trimer in less than 10% yield (based on previously reported spectroscopic data).

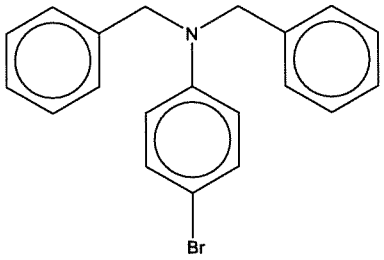


44

***N*-benzyl-4-bromoaniline (44). CAS 2879-83-6**

This compound has been previously reported by Dains *et al.*[78] The method for which its preparation is based upon has been previously reported by Bordwell *et al.*[76] To an oven-dried 15 mL 3-neck round bottom flask containing a spin bar was added potassium hydride slurry (30% KH/mineral spirits) (168 mg slurry, 1.26 mmol actual reagent). The

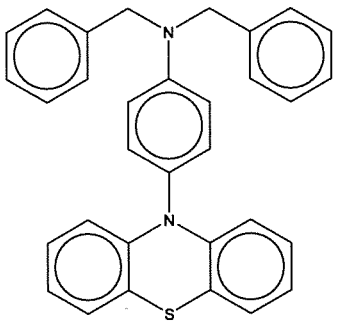
mineral spirits were removed with petroleum ether washes (3 x 3 mL freshly distilled from CaH_2), and to the potassium hydride was immediately added *N,N*-dimethylformamide (DMF) (0.5 mL). A solution of 4-bromoaniline (**24**) (200 mg, 1.16 mmol) in DMF (2.8 mL) was added, and the reaction was stirred under nitrogen for 20 minutes. To the reaction was then added benzyl bromide (**43**) (0.3 mL, 3.99 mmol) dropwise, and the reaction was stirred for 48 hours. The reaction was quenched with de-ionized water (5 mL, dropwise), stirred for 20 minutes, extracted with CH_2Cl_2 (3x15 mL), washed with de-ionized water, and washed with brine. The combined organics were dried with magnesium sulfate, filtered through fritted glass, and the filtrate was concentrated under vacuum. Purification by gravity chromatography (silica, minimal CH_2Cl_2 to dissolve crude, gradient pet ether, 5% Et_2O/pet ether, 10% Et_2O/pet ether, 20% Et_2O/pet ether, 40% Et_2O/pet ether) afforded 100 mg (25.3%) of **44** as a yellow solid: TLC R_f 0.14 (10% Et2O/pet ether), ^1H NMR (300 MHz, $CDCl_3$) δ 7.32 (m, 5H), δ 7.21 (d, 2H), δ 6.59 (d, 2H), δ 4.63 (bs, 2H), δ 1.55 (s, 1H).



45

***N,N*-dibenzyl-4-bromoaniline (45). CAS 65145-14-4**

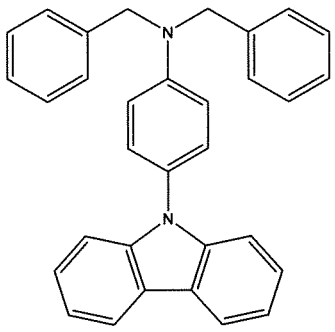
This compound, as well as the method for which its preparation is based upon, has been previously reported by Etienne.[79] To an oven-dried 50 mL 3-neck round bottom flask containing a spin bar was added potassium hydride slurry (30% KH/mineral spirits) (672 mg slurry, 5.04 mmol actual reagent). The mineral spirits were removed with hexane washes (3 x 5 mL), and to the potassium hydride was immediately added *N,N*-dimethylformamide (DMF) (2.0 mL). A solution of 4-bromoaniline (**24**) (800 mg, 4.64 mmol) in DMF (11.2 mL) was added, and the reaction was stirred under nitrogen for 20 minutes. To the reaction was then added benzyl bromide (**43**) (1.2 mL, 15.96 mmol) dropwise, and the resulting opaque orange reaction was stirred for 48 hours. The reaction was quenched with de-ionized water (minimal number of drops to initiate precipitation of a white solid) and stirred for 60 minutes. The precipitate was filtered, rinsed with cold de-ionized water, rinsed with cold hexane, and pumped to afford 1.25 g (78.6%) of **45** as a fluffy yellowish-white solid: mp 122-126 °C; TLC R_f 0.40 (5% Et2O/pet ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (m, 12H), δ 6.59 (d, 2H), δ 4.63 (s, 4H).



49

***N,N*-dibenzyl-4-(10*H*-phenothiazin-10-yl)aniline (49)**

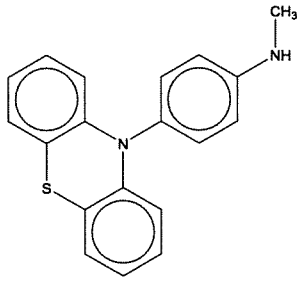
This compound does not appear to have been previously reported. The method for which its preparation is based upon has been previously reported by Ferreira *et al.*[77] To a 20 mL pressure flask containing a spin bar and *N,N*-dibenzyl-4-bromoaniline (**45**) (100 mg, 0.280 mmol) was added 10*H*-phenothiazine (**8**) (56 mg, 0.280 mmol), bis(dibenzylideneacetone)palladium(0) (7 mg, 0.012 mmol), 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (BINAP) (47 mg, 0.076 mmol), cesium carbonate (1.32 g, 4.04 mmol), and toluene (9 mL). The flask was purged with argon, sealed, and refluxed for 120 hours between 140-160 °C. The reaction was gravity filtered, chased with CH_2Cl_2, and the filtrate concentrated under vacuum to afford 433 mg (90.0%) of **49** as an off-white solid: mp 156-160 °C; TLC (10% ET_2O/petroleum ether) R_f 0.42; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.38 – 7.29 (m, 8H), δ 7.12 (d, 2H), δ 6.95 – 6.76 (m, 10H), δ 6.25 (d, 2H).



50

***N,N*-dibenzyl-4-(9*H*-carbazol-9-yl)aniline (50). CAS 53167-73-0**

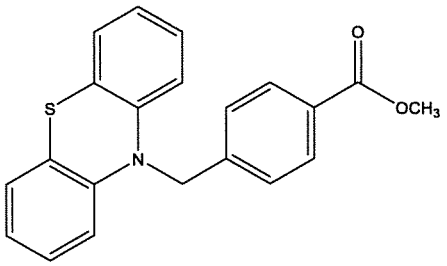
This compound has been previously reported by Janssens *et al.*[80] The method for which its preparation is based upon has been previously reported by Ferreira *et al.*[77] To a 20 mL pressure flask containing a spin bar and toluene (1 mL) was added cesium carbonate (1.32 g, 0.178 mmol), followed by 9*H*-carbazole (15 mg, 0.090 mmol), and the reaction was stirred at room temperature for 20 minutes. To the reaction was added palladium (II) acetate (2.5 mg, 0.011 mmol), 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (BINAP) (4 mg, 0.006 mmol), and *N,N*-dibenzyl-4-bromoaniline (**45**) (30 mg, 0.084 mmol). The flask was purged with nitrogen, sealed, and refluxed for 40 hours at 140 °C. The reaction was gravity filtered through silica, chased with 5% CH_2Cl_2/5% EtOAc/petroleum ether, and the filtrate concentrated under vacuum. Purification by chromatography (silica, gradient 10% Et_2O/pet ether, 20% Et2O/pet ether) afforded 28 mg (75.7%) of **50** as a dark yellow oil: TLC R_f 0.45 (10% Et2O/pet ether); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.36 (m, 4H), δ 7.30 (m, 6H), δ 7.13 (d, 2H), δ 6.97 (d, 2H), δ 6.89 (d, 2H), δ 6.83 (d, 2H), δ 6.77 (d, 2H).



84

_N_-methyl-4-(9_H_-phenothiazin-10-yl)aniline (84). CAS 58737-01-2

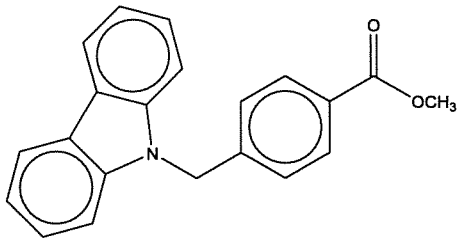
This compound has been previously reported by Clarke *et al.*[72] The method for which its preparation is based upon has been previously reported by Ferreira *et al.*[77] To a 20 mL pressure flask containing a spin bar and xylene (2.0 mL) was added 4-bromo-*N*-methylaniline (**45**) (100 μL, 0.799 mmol) was added tri-*tert*-butylphosphine (33.3 μL, 0.137 mmol), palladium(II) acetate (6.7 mg, 0.029 mmol), 10*H*-phenothiazine (**8**) (333 mg, 1.67 mmol), and sodium *tert*-butoxide (350 mg, 3.65 mmol). The walls were rinsed with xylene (8 mL) and argon was bubbled into solution for 10 minutes. The flask was sealed and refluxed for 120 hours at 120 °C. The reaction was gravity filtered through silica, chased with CH_2Cl_2, and the filtrate concentrated under vacuum to afford **84** as brown crystals. Column chromatography (silica, 10% Et_2O/pet ether) removed unreacted phenothiazine: TLC (30% Et2O/pet ether) R_f 0.27; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.02 (m, 4H), δ 6.97 (d, 2H), δ 6.86 (t, 2H), δ 6.79 (t, 2H), δ 6.25 (bs, 1H), δ 6.23 (d, 2H), δ 1.25 (bs, 3H).



88

Methyl-4-(10*H*-phenothiazin-10-yl)aniline (88). CAS 1638542-58-1

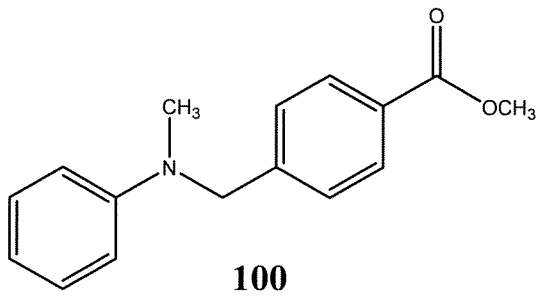
This compound has been previously reported by Levi *et al.*[81] The method for which its preparation is based upon has been previously reported by Bordwell *et al.*[76] To an oven-dried 50 mL 3-neck round bottom flask containing a spin bar was added potassium hydride slurry (30% KH/mineral spirits) (67 mg slurry, 0.503 mmol actual reagent). The mineral spirits were removed with petroleum ether washes (3 x 5 mL), and to the potassium hydride was immediately added *N*,*N*-dimethylformamide (DMF) (0.5 mL). Phenothiazine (**8**) (80 mg, 0.464 mmol) was then added, followed by methyl 4-(bromomethyl) benzoate (**87**) (92 mg, 0.402 mmol). The flask was rinsed with DMF (0.5 mL), and the reaction was stirred under argon for 7 days. The reaction was quenched with de-ionized water dropwise and stirred for 10 minutes. The quenched reaction was extracted with petroleum ether (3 x 5 mL) and the combined organics dried over sodium hydrosulfite, filtered, and concentrated under vacuum to afford greater than 95% conversion (as determined by proton NMR; residual DMF present) to **88** as a burgundy oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41 (d, 2H), δ 7.09 (d, 2H), δ 6.96 (m, 2H), δ 6.88 (m, 2H), δ 6.76 (bd, 2H), δ 6.61 (d, 2H), δ 5.70 (s, 2H), δ 3.85 (s, 3H).



99

Methyl-4-(9*H*-phenothiazin-9-yl)methyl)benzoate (99). CAS 468066-07-1P

This compound has been previously reported by Taguchi *et al.*[82] The method for which its preparation is based upon has been previously reported by Bordwell *et al.*[76] To an oven-dried 20 mL round bottom flask containing a spin bar was added potassium hydride slurry (30% KH/mineral spirits) (268 mg slurry, 2.01 mmol actual reagent). The mineral spirits were removed with petroleum ether (3 x 3 mL), and to the potassium hydride was immediately added *N*,*N*-dimethylformamide (DMF) (2 mL). Carbazole (268 mg, 1.60 mmol) was then added, followed by methyl 4-(bromomethyl) benzoate (**87**) (368 mg, 1.61 mmol). The flask was rinsed with DMF (2 mL), and the reaction was stirred under argon for 48 hours. The reaction was quenched with de-ionized water dropwise to initiate precipitate formation, and the quenched reaction was stirred at room temperature for 10 minutes. The precipitate was filtered through fritted glass, rinse with cold de-ionized water, and concentrated under vacuum to afford 272 mg (54.0%) of **99** as a white solid: TLC R_f 0.61 (50% Et2O/pet ether); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.18 (d, 2H), δ 7.94 (d, 2H), δ 7.43 (d, 2H), δ 7.32 (m, 4H), δ 7.19 (d, 2H), δ 5.59 (s, 2H), δ 3.88 (s, 3H).

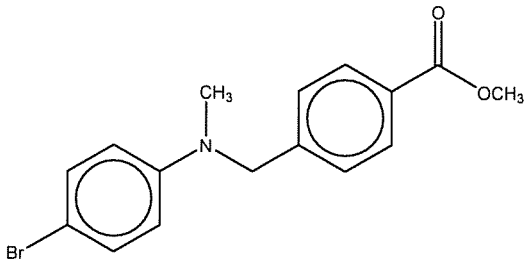


100

Methyl 4-((methyl(phenyl)amino)methyl)benzoate (100). CAS 398469-98-2

This compound has been previously reported by Hartwig *et al.*[83] The method for which its preparation is based upon has been previously reported by Bordwell *et al.*[76] To an oven-dried 5 mL conical vial containing a spin bar was added potassium hydride slurry (30% KH/mineral spirits) (67 mg, 0.503 mmol actual reagent). The mineral spirits were removed with hexane washes (3 x 4 mL), and to the potassium hydride was immediately added *N,N*-dimethylformamide (DMF) (0.5 mL). The suspension was stirred at room temperature, and to it was added a solution of *N*-methylaniline (50 μL, 0.465 mmol) in DMF (1 mL), followed immediately by methyl 4-bromomethylbenzoate (100 mg, 0.437 mmol). The reaction was stirred at room temperature for 48 hours, upon which time a green tint color had formed. [Note: the reaction requires at least 40 hours to obtain an optimized yield, however it can stir for at least 72 hours without the observance of a significant reduction in yield or purity.] The reaction was quenched with 2 mL acetic acid/de-ionized water (1:1 v/v) and stirred for 5 minutes. A spatula of sodium bicarbonate was then added to neutralize unreacted acetic acid. The contents of the vial were transferred to a separatory funnsxel and extracted with diethyl ether (3 x 10 mL). The combined organic layers were washed with brine (2 x 10 mL), washed with de-ionized water (2 x 10 mL), dried over magnesium sulfate, filtered, and the filtrate was

concentrated to afford 71 mg (75%) of **100** as a yellowish-green oil: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.87 (d, 2H), δ 7.20 (d, 2H), δ 7.11 (m, 2H), δ 6.62 (d, 2H), δ 4.45 (s, 2H), δ 3.78 (s, 3H), δ 2.92 (s, 3H).

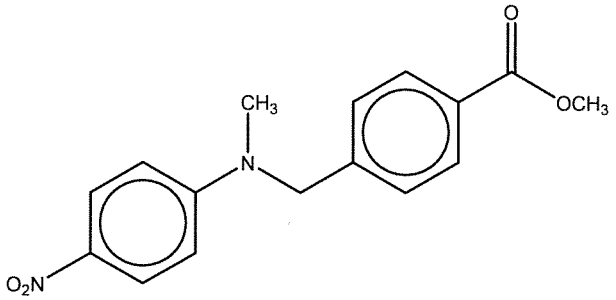


101

Methyl-4-(((4-bromophenyl)(methyl)amino)methyl)benzoate (101).

This compound does not appear to have been previously reported. The method for which its preparation is based upon has been previously reported by Bordwell *et al.*[76] To an oven-dried 5 mL conical vial containing a spin bar was added potassium hydride slurry (30% KH/mineral spirits) (67 mg, 5.583 mmol). The mineral spirits were removed with petroleum ether washes (3 x 4 mL), and to the potassium hydride was immediately added *N,N*-dimethylformamide (DMF) (2 mL). The suspension was stirred at room temperature, and to it was added *N*-methyl-4-bromoaniline (50 μL, 0.400 mmol), followed immediately by methyl 4-bromomethylbenzoate (**87**) (100 mg, 0.437 mmol). The reaction was stirred at room temperature for 48 hours, upon which time a blue color had formed. [Note: the reaction requires at least 40 hours to obtain an optimized yield, however it can stir for at least 72 hours without the observance of a significant reduction in yield or purity.] The reaction was quenched with 2 mL acetic acid/de-ionized water (1:1 v/v) and stirred for 5 minutes. A spatula of sodium bicarbonate was then added to neutralize unreacted acetic acid. The contents of the vial were transferred to a separatory

funnel and extracted with diethyl ether (3 x 10 mL). The combined organic layers were washed with brine (2 x 10 mL), washed with de-ionized water (2 x 10 mL), dried over magnesium sulfate, filtered, and the filtrate was concentrated to afford 87% conversion (as determined by proton NMR) to **101** as a green oil: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.88 (d, 2H), δ 7.15 (d, 2H), δ 7.13 (d, 2H), δ 6.45 (d, 2H), δ 4.41 (s, 2H), δ 3.80 (s, 3H), δ 1.97 (s, 3H).

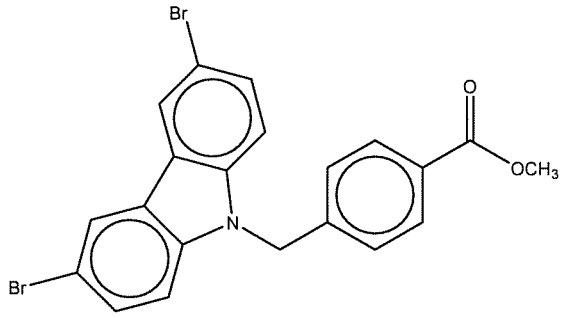


102

Methyl 4-((methyl(4-nitrophenyl)amino)methyl)benzoate (102)

This compound does not appear to have been previously reported. The method for which its preparation is based upon has been previously reported by Bordwell *et al.*[76] To an oven dried 5 mL conical vial containing a spin bar was added potassium hydride slurry (30% KH/mineral spirits) (67 mg, 5.583 mmol). The mineral spirits were removed with petroleum ether washes (3 x 4 mL), and to the potassium hydride was immediately added *N,N*-dimethylformamide (DMF) (2 mL). The suspension was stirred at room temperature, and to it was added *N*-methyl-4-nitroaniline (60 mg, 0.395 mmol), followed immediately by methyl 4-bromomethylbenzoate (**87**) (100 mg, 0.437 mmol). The reaction was stirred at room temperature for 48 hours, upon which time a blue color had formed. [Note: the reaction requires at least 40 hours to obtain an optimized yield,

however it can stir for at least 72 hours without the observance of a significant reduction in yield or purity.] The reaction was quenched with 2 mL acetic acid/de-ionized water (1:1 v/v) and stirred for 5 minutes. A spatula of sodium bicarbonate was then added to neutralize unreacted acetic acid. The contents of the vial were transferred to a separatory funnel and extracted with diethyl ether (3 x 10 mL). The combined organic layers were washed with brine (2 x 10 mL), washed with de-ionized water (2 x 10 mL), dried over magnesium sulfate, filtered, and the filtrate was concentrated to afford 56% conversion (as determined by proton NMR) to **102** as a yellow solid: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.98 (d, 2H), δ 7.89 (d, 2H), δ 7.14 (d, 2H), δ 6.42 (d, 2H), δ 4.63 (s, 2H); δ 3.82 (s, 3H); δ 2.82 (s, 3H).

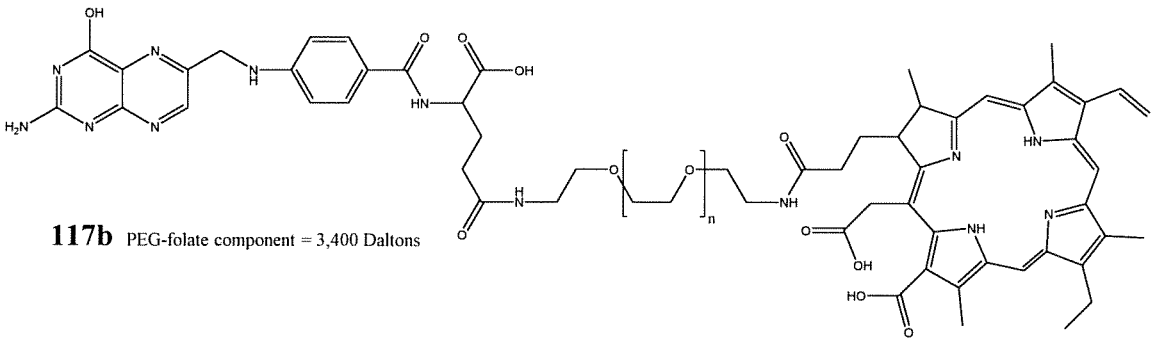


105

Methyl 4-((3,6-dibromo-9*H*-carbazol-9-yl)methylbenzoate (105)

This compound does not appear to have been previously reported. The method for which its preparation is based upon has been previously reported by Bordwell *et al.*[76] To a 20 mL pressure flask containing a spin bar and methyl-4-(9*H*-phenothiazin-9-yl)methyl)benzoate (**99**) (100 mg, 0.317 mmol) was added CH_2Cl_2 (1.5 mL), followed by sodium bicarbonate (53 mg, 0.631 mmol). In a separate heart-shaped flask containing CH_2Cl_2 (0.75 mL) was added bromine (30 μL, 0.581 mmol). The bromine solution was

transferred dropwise to the pressure tube, with residual bromine rinsed from the walls of the heart-shape flask into the pressure tube using CH_2Cl_2 (0.75 mL). The reaction was stirred in the dark at room temperature for 72 hours. The reaction was transferred to a separatory funnel with de-ionized water, extracted with CH_2Cl_2 (3 x 5 mL), and washed with de-ionized water (3 x 15 mL). The combined organics were dried with sodium sulfate, filtered through filter paper, and concentrated under vacuum to afford 115 mg (76.7%) of **105** as fluffy yellow crystals: mp 122-124 °C (use of a drying oven for several hours resulted in the crystals changing color from yellow to blue, but did not change the melting point, indicating that decomposition had not occurred); TLC R_f 0.48 (50% Et_2O/petroleum ether); ^1H NMR (300 MHz, $CDCl_3$) δ 8.20 (s, 2H), δ 7.94 (d, 2H), δ 7.53 (d, 2H), δ 7.19 (d, 2H), δ 7.12 (d, 2H), δ 5.52 (s, 2H), δ 3.88 (s, 3H).

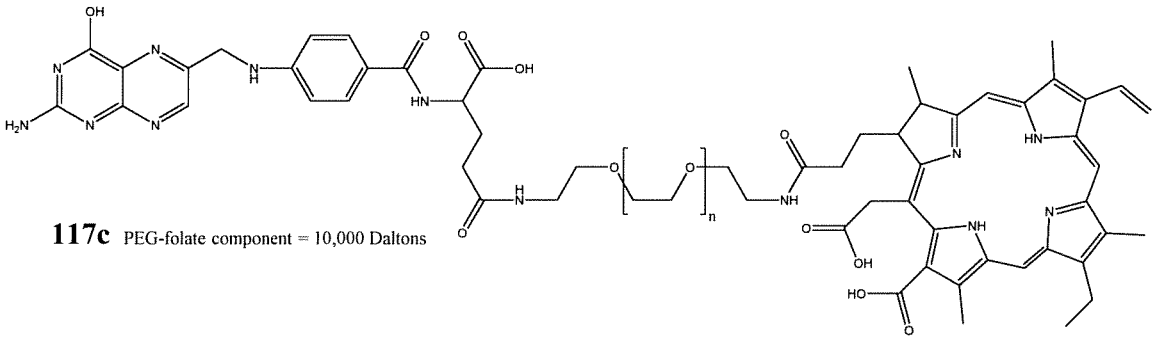


117b PEG-folate component = 3,400 Daltons

7-(1-(4-(((2-amino-4-hydroxypteridin-6-yl)methyl)amino)phenyl)-3-carboxy-1,6,17-trioxo-10,13-dioxa-2,7,16-triazanonadecan-19-yl)-5-(carboxymethyl)-18-ethyl-2,8,12,17-tetramethyl-13-vinyl-7*H*,8*H*-porphyrin-3-carboxylic acid (117b)

This compound has not been reported in the literature. To an oven dried 5 mL conical vial containing a spin vane was added 6.0 mg (0.292 μmol) PEG 20000 Folate, 0.8 mg

(1.34 μmol) Chlorin e6, and 1.0 mL dichloromethane. The suspension was stirred at room temperature, during which 1 capillary tube-size drop of trimethylamine was added, followed by 0.6 mg dicyclohexylcarbodiimide, 0.4 mg hydroxybenzyltriazole (HOBT), and 1.0 mL dichloromethane. The dark green (nearly black) reaction was stirred under argon in the dark at room temperature for 96 hours. The reaction was then concentrated under vacuum, dissolved in minimal methanol, and passed through a column consisting of sephadex size-exclusion gel (10 g, G10). Fractions of approximately 4-5 mL were collected, and those not containing residual Chlorin e6 (as determined by TLC) were combined, concentrated under vacuum to afford 1.5 mg (25%) of **117b** as a green oil: TLC *Rf* 0.16 (3:1 H_2O, MeOH); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.00-3.60 (vbm), δ 3.38 (bs), δ 1.80 (bs), δ 1.24 (bs); UV/VIS λ 350 nm, 400 nm, 510 nm, 660 nm.

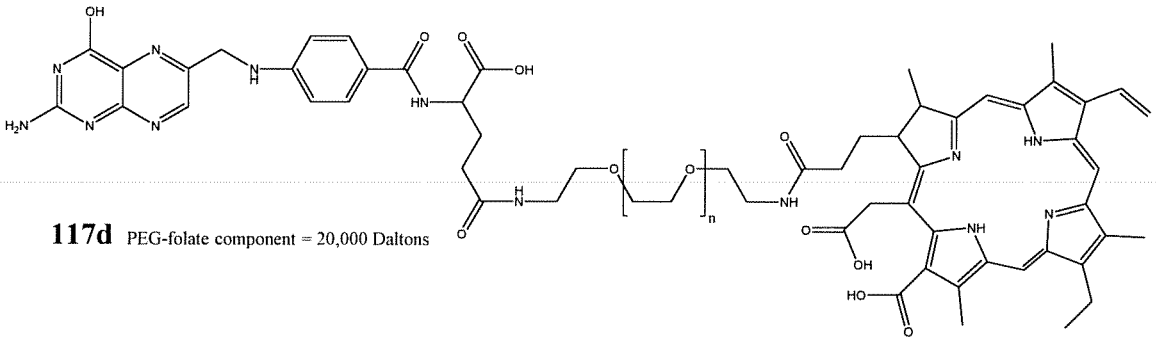


117c PEG-folate component = 10,000 Daltons

7-(1-(4-(((2-amino-4-hydroxypteridin-6-yl)methyl)amino)phenyl)-3-carboxy-1,6,17-trioxo-10,13-dioxa-2,7,16-triazanonadecan-19-yl)-5-(carboxymethyl)-18-ethyl-2,8,12,17-tetramethyl-13-vinyl-7*H*,8*H*-porphyrin-3-carboxylic acid (117c)

This compound has not been reported in the literature. To an oven dried 5 mL conical vial containing a spin vane was added 6.0 mg (0.292 μmol) PEG 20000 Folate, 0.8 mg

(1.34 μmol) Chlorin e6, and 1.0 mL dichloromethane. The suspension was stirred at room temperature, during which 1 capillary tube-size drop of trimethylamine was added, followed by 0.6 mg dicyclohexylcarbodiimide, 0.4 mg hydroxybenzyltriazole (HOBT), and 1.0 mL dichloromethane. The dark green (nearly black) reaction was stirred under argon in the dark at room temperature for 96 hours. The reaction was then concentrated under vacuum, dissolved in minimal methanol, and passed through a column consisting of sephadex size-exclusion gel (10 g, G10). Fractions of approximately 4-5 mL were collected, and those not containing residual Chlorin e6 (as determined by TLC) were combined, concentrated under vacuum to afford 1.5 mg (25%) of **117c** as a green oil: TLC *Rf* 0.16 (3:1 H_2O, MeOH); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.00-3.60 (vbm), δ 3.38 (bs), δ 1.80 (bs), δ 1.24 (bs); UV/VIS λ 350 nm, 400 nm, 510 nm, 660 nm.

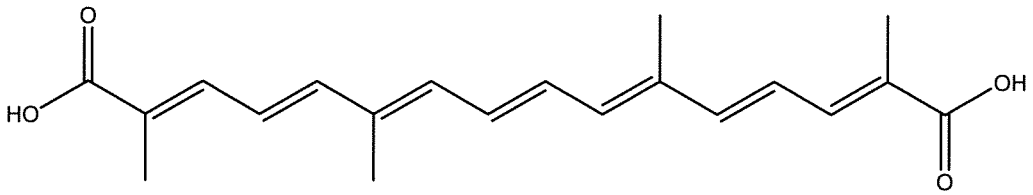


117d PEG-folate component = 20,000 Daltons

7-(1-(4-(((2-amino-4-hydroxypteridin-6-yl)methyl)amino)phenyl)-3-carboxy-1,6,17-trioxo-10,13-dioxa-2,7,16-triazanonadecan-19-yl)-5-(carboxymethyl)-18-ethyl-2,8,12,17-tetramethyl-13-vinyl-7*H*,8*H*-porphyrin-3-carboxylic acid (117d)

This compound has not been reported in the literature. To an oven dried 5 mL conical vial containing a spin vane was added 6.0 mg (0.292 μmol) PEG 20000 Folate, 0.8 mg

(1.34 μmol) Chlorin e6, and 1.0 mL dichloromethane. The suspension was stirred at room temperature, during which 1 capillary tube-size drop of trimethylamine was added, followed by 0.6 mg dicyclohexylcarbodiimide, 0.4 mg hydroxybenzyltriazole (HOBT), and 1.0 mL dichloromethane. The dark green (nearly black) reaction was stirred under argon in the dark at room temperature for 96 hours. The reaction was then concentrated under vacuum, dissolved in minimal methanol, and passed through a column consisting of sephadex size-exclusion gel (10 g, G10). Fractions of approximately 4-5 mL were collected, and those not containing residual Chlorin e6 (as determined by TLC) were combined, concentrated under vacuum to afford 1.5 mg (25%) of **117d** as a green oil: TLC *Rf* 0.16 (3:1 H_2O, MeOH); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.00-3.60 (vbm), δ 3.38 (bs), δ 1.80 (bs), δ 1.24 (bs); UV/VIS λ 350 nm, 400 nm, 510 nm, 660 nm.

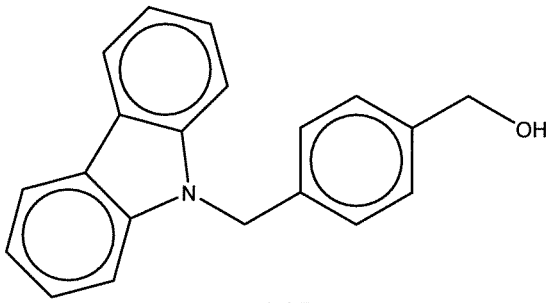


130

2E,4E,6E,8E,10E,12E,14E)-2,6,11,15-tetramethylhexadeca-2,4,6,8,10,12,14-heptaenedioic acid (crocetin diacid) (130). CAS 27876-94-4

This compound has been previously reported by Kayser.[84] The method for which its preparation is based upon has been previously reported by Glass.[85] To a 15 mL round bottom flask containing a spin bar and bis-β-D-glucosyl (crocetin) (**130**) (50 mg, 0.077 mmol) was added de-ionized water (2 mL), followed by mortar-ground sodium hydroxide

pellets (60 mg, 1.5 mmol), and the suspension was refluxed for 20 minutes via bunsen burner. The contents of the flask were transferred to a glass centrifuge tube with de-ionized water and acidified with dropwise addition of sulfuric acid (1.0 M) to produce an orangish-red precipitate. The suspension was centrifuged for 10 minutes, and the aqueous layer was removed by pipet to afford greater than 95% conversion (as determined by proton NMR; residual water present) to **130** as a red solid: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.73 (d, 4H), 7.36 – 7.01 (m, 16H, includes $CHCl_3$), δ 2.48 (d, 6H), δ 2.42 (d, 6H).

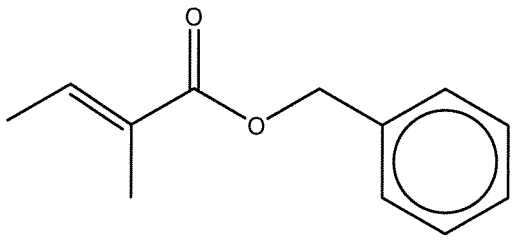


145

(4-((9*H*-carbazol-9-yl)methyl)phenyl)methanol (145). CAS 1367364-47-3

This compound has been previously reported by Du *et al.*[86] The method for which its preparation is based upon has been previously reported by Nystrom *et al.*[87] To an oven-dried 5 mL round bottom flask containing a spin bar and methyl-4-(9*H*-phenothiazin-9-yl)methyl)benzoate (**99**) (126 mg, 0.400 mmol) was added tetrahydrofuran (THF) (3 mL). The flask was cooled to 0° C, and to it was added a lithium aluminum hydride (LAH)/THF solution (1.4 mL, 1.0 M). The reaction was allowed to warm to room temperature while stirring for 48 hours. The reaction was transferred to an Erlenmeyer using diethyl ether (5 mL) and quenched with dropwise addition of aqueous ammonium

hydroxide (2 M), producing a suspension. The ether layer was decanted into a clean Erlenmeyer. Ether extracts and ammonium hydroxide washes with subsequent decanting were repeated twice more, followed by a de-ionized water wash. The combined ether layers were dried over magnesium sulfate, filtered through fritted glass, and concentrated under vacuum to afford 83 mg (72.8%) of **145** as a white solid: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.12 (d, 2H), δ 8.06 (d, 2H), δ 7.36 (m, 2H), δ 7.19 (m, 4H), δ 7.13 (d, 2H), δ 5.46 (s, 2H), δ 4.59 (s, 2H), δ 2.32 (s, 1H).

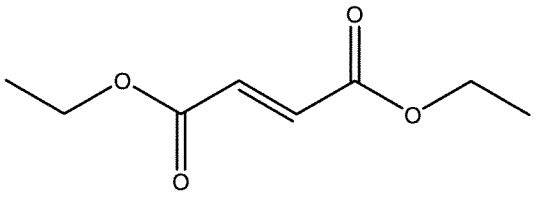


155

Benzyl (E)-2-methylbut-2-enoate (155). CAS 37526-88-8

This compound has been previously reported by Pilsov *et al.*[88] The method for which its preparation is based upon has been previously reported by Durst *et al.*[89] To a 50 mL oven-dried round bottom flask containing a spin bar and (E)-2-methylbut-2-enoic acid (tiglic acid) (**154**) (2.00 g, 0.02 mmol) was added benzyl alcohol (2.0 mL, 0.019 mmol) and toluene (5 mL), followed by sulfuric acid (2 drops, 18.0 M). The flask was fitted with a Dean-Stark trap, and the collecting arm was filled with toluene. The reaction was stirred for 12 hours at reflux, then cooled to room temperature. The acid catalyst was neutralized with sodium carbonate (1 spatula) and stirred for 5 minutes. The reaction was extracted with diethyl ether (2 x 5 mL), washed with de-ionized water (2 x 5 mL), and the

combined organics were dried over magnesium sulfate, filtered through fritted glass, and concentrated under vacuum to afford 3.59 g (92.2%) of **155** as a golden brown oil: ^{1}H-NMR (300 MHz, $CDCl_3$).



166

Diethyl fumarate (166). CAS 623-91-6

This compound has been previously reported by Reiter *et al.*[90] The method for which its preparation is based upon has been previously reported by Amer *et al.*[91] To an oven-dried 25 mL round bottom flask containing a spin bar and toluene (6.6 mL) was added ethyl ortho formate (3.3 mL, 0.020 mmol), (E)-but-2-ene-1,4-dioic acid (fumaric acid) (116 mg, 1.00 mmol), Dowex 50WX8 hydrogen form (400 mesh) acidic ion-exchange resin (1.00 g), and sulfuric acid (1 drop, 18.0 M). The reaction was refluxed at 100 °C for 24 hours. The reaction was allowed to cool to room temperature, filtered through fritted glass, and the filtrate was extracted with diethyl ether (2 x 10 mL), washed with de-ionized water (2 x 10 mL), washed with aqueous brine. The combined organics were dried over magnesium sulfate, filtered through fritted glass, and concentrated to afford 118 mg (68.6%) of **166**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 6.77 (s, 2H), δ 4.17 (q, 4H), δ 1.24 (t, 6H).

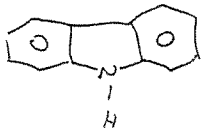
APPENDIX A

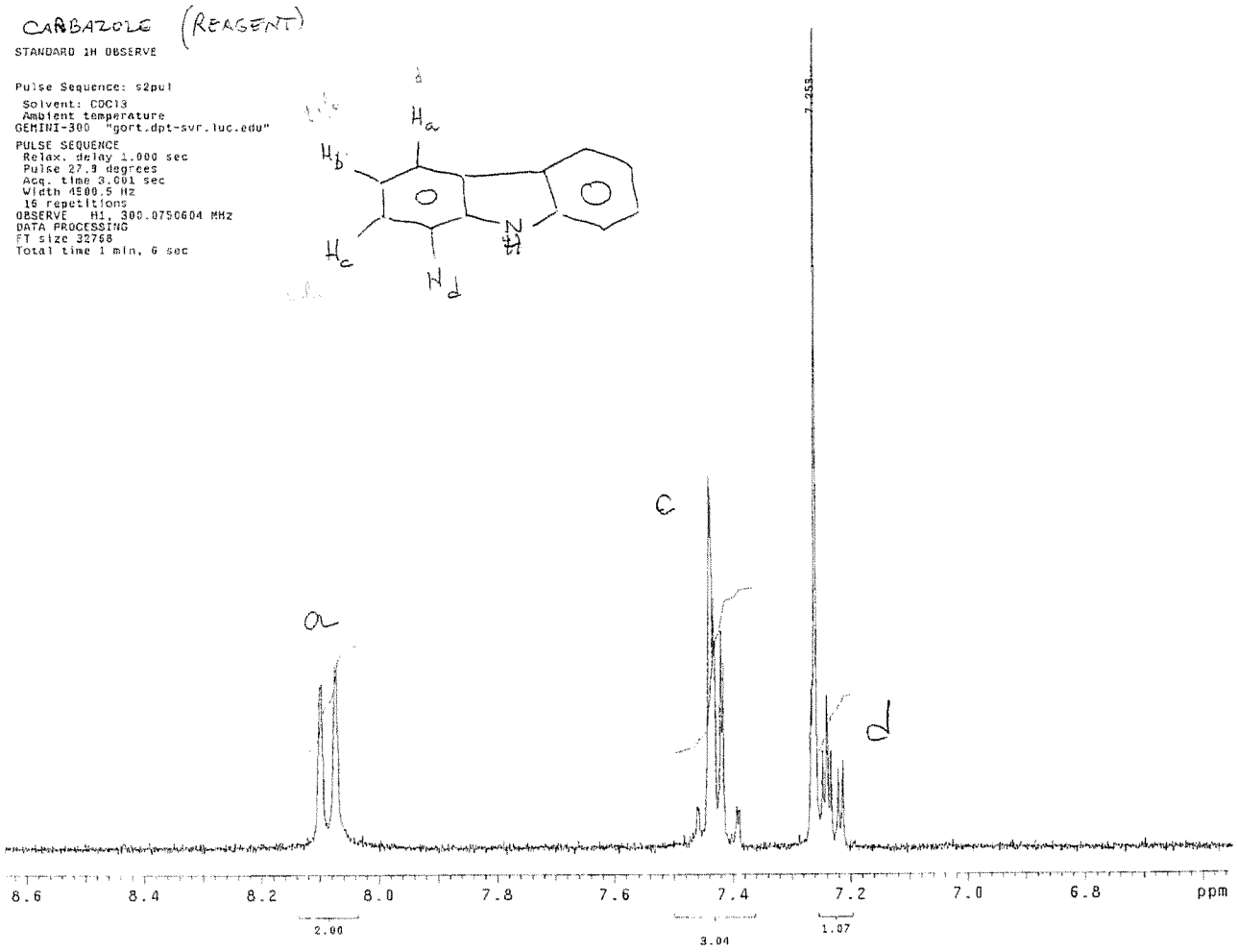
SPECTRA

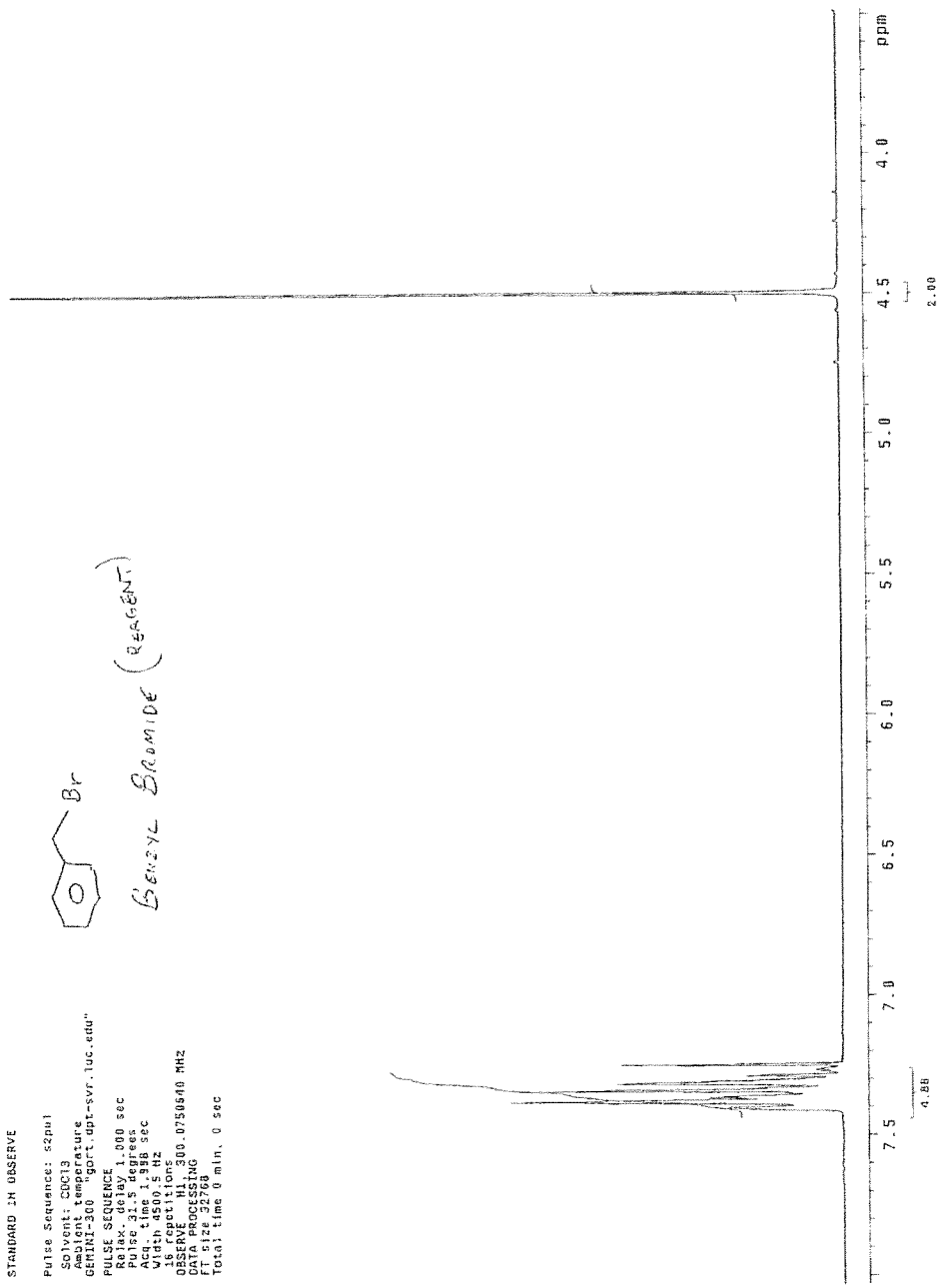
CARBAZOLE (REAGENT)

STANDARD 1H OBSERVE

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750604 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec



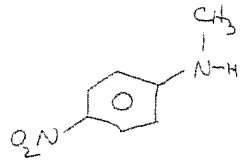

CARBAZOLE (REAGENT)
STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750604 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
Ha
Hb
Hc
Hd
N
7.255
a
c
d
8.6
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
ppm
2.00
3.04
1.07

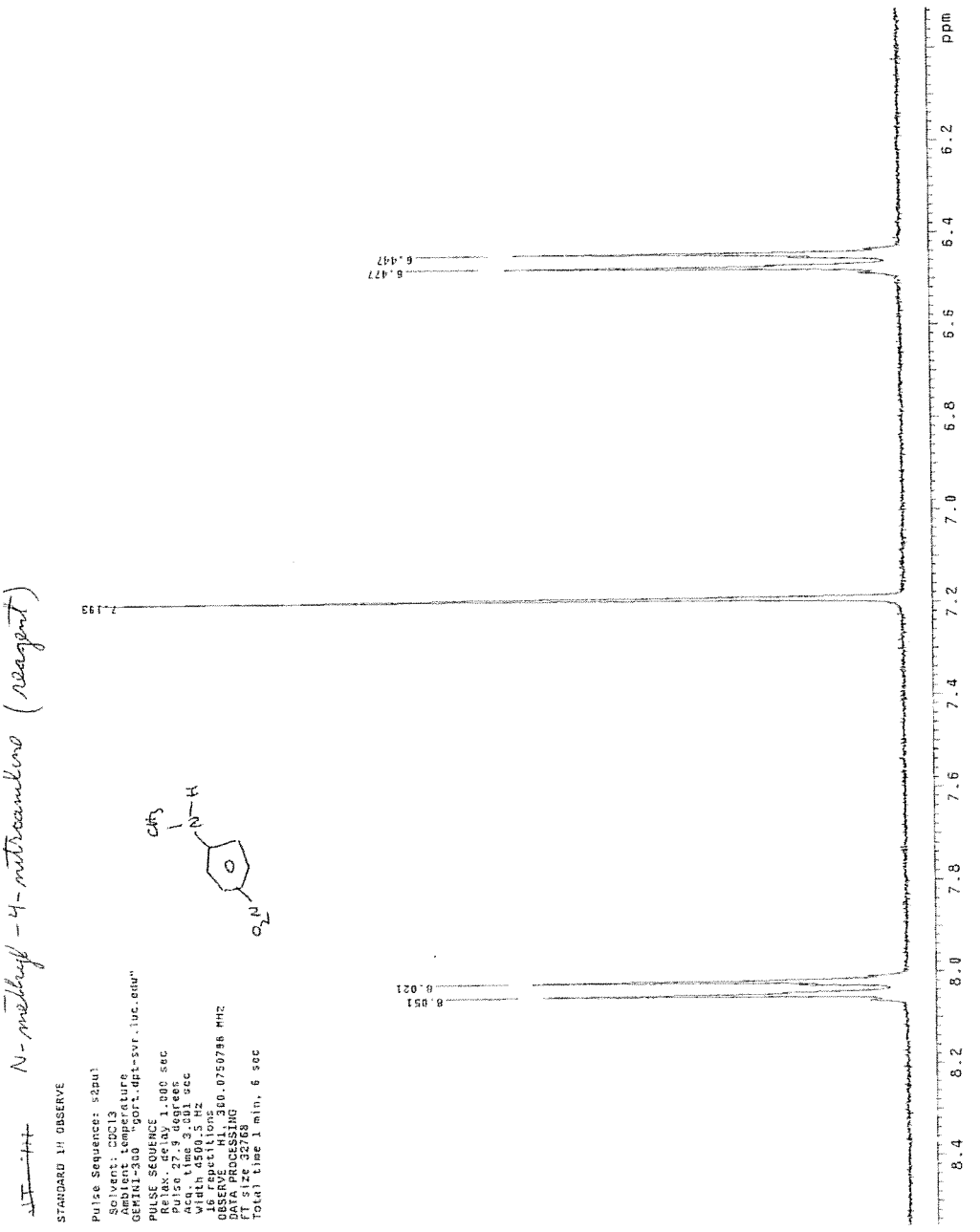

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.upt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 31.5 degrees
Acq. time 1.998 sec
Width 4501.5 Hz
16 repetitions
OBSERVE H1, 300.0750440 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 0 sec
Br
Benzyl Bromide (Reagent)
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
ppm
4.86
2.00

N-Methyl-4-nitroaniline (reagent)

STANDARD 1H OBSERVE

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750796 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec

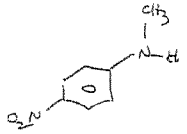


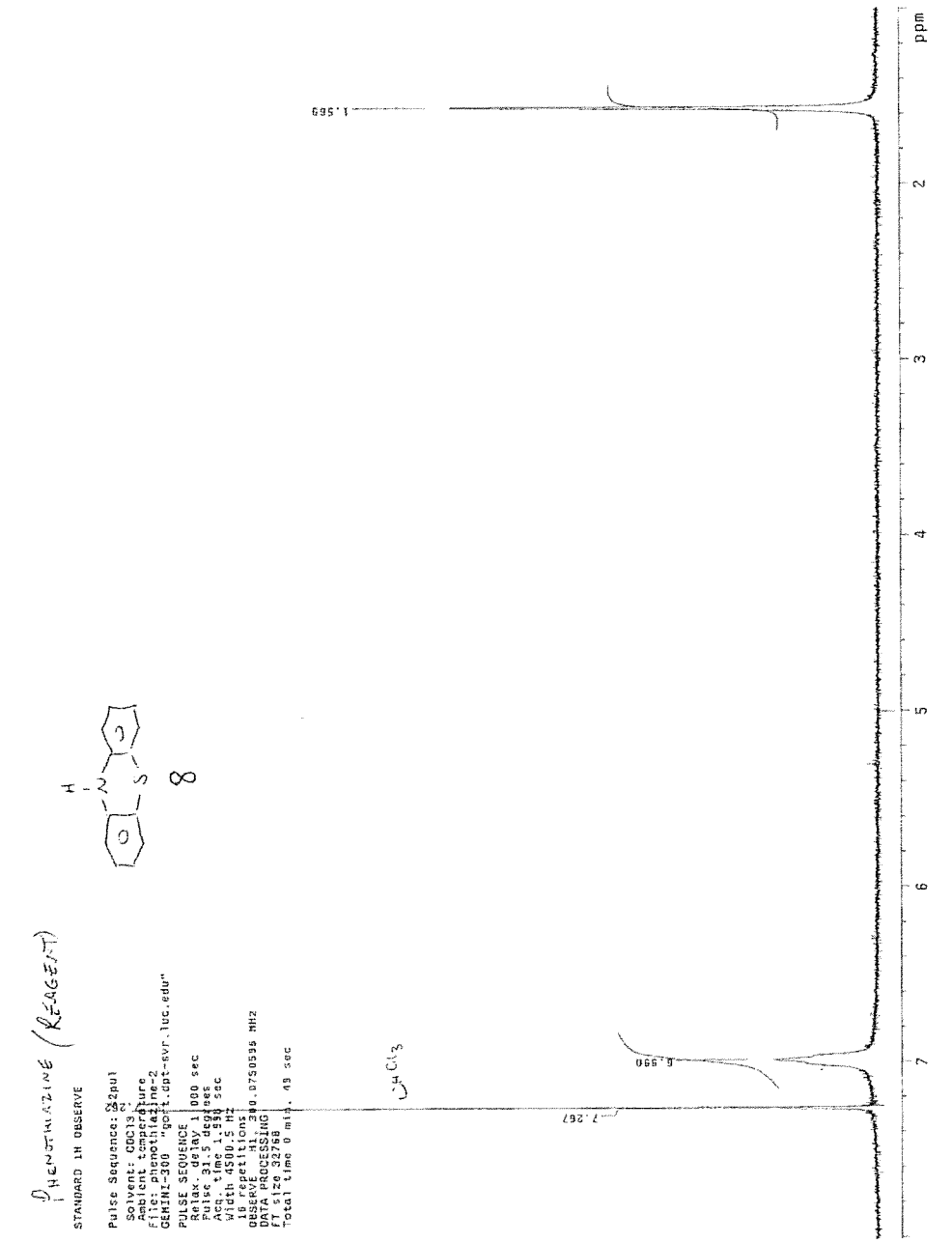

N-methyl-4-nitroaniline (reagent)
STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750786 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
8.051
8.021
6.477
6.447
2.193
8.4
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
ppm

N-methyl-4-nitroaniline (reagent)

STANDARD 1H OBSERVE

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750796 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec



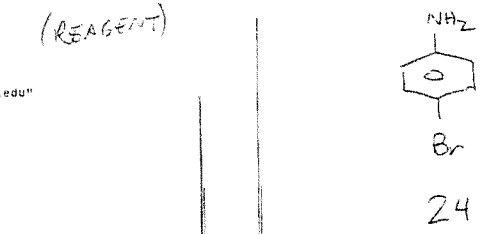

PHENOTHIAZINE (REAGENT)
8
STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: phenothiazine-2
GEMINI-300 "gofl.dpt-rvr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 31.5 degrees
Acq. time 1.999 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750596 MHz
DATA PROCESSING
FT size 32768
Total time 0 min. 49 sec
7.267
CHCl3
6.990
1.569
7
6
5
4
3
2
ppm

4-BROMOANILINE

STANDARD 1H OBSERVE

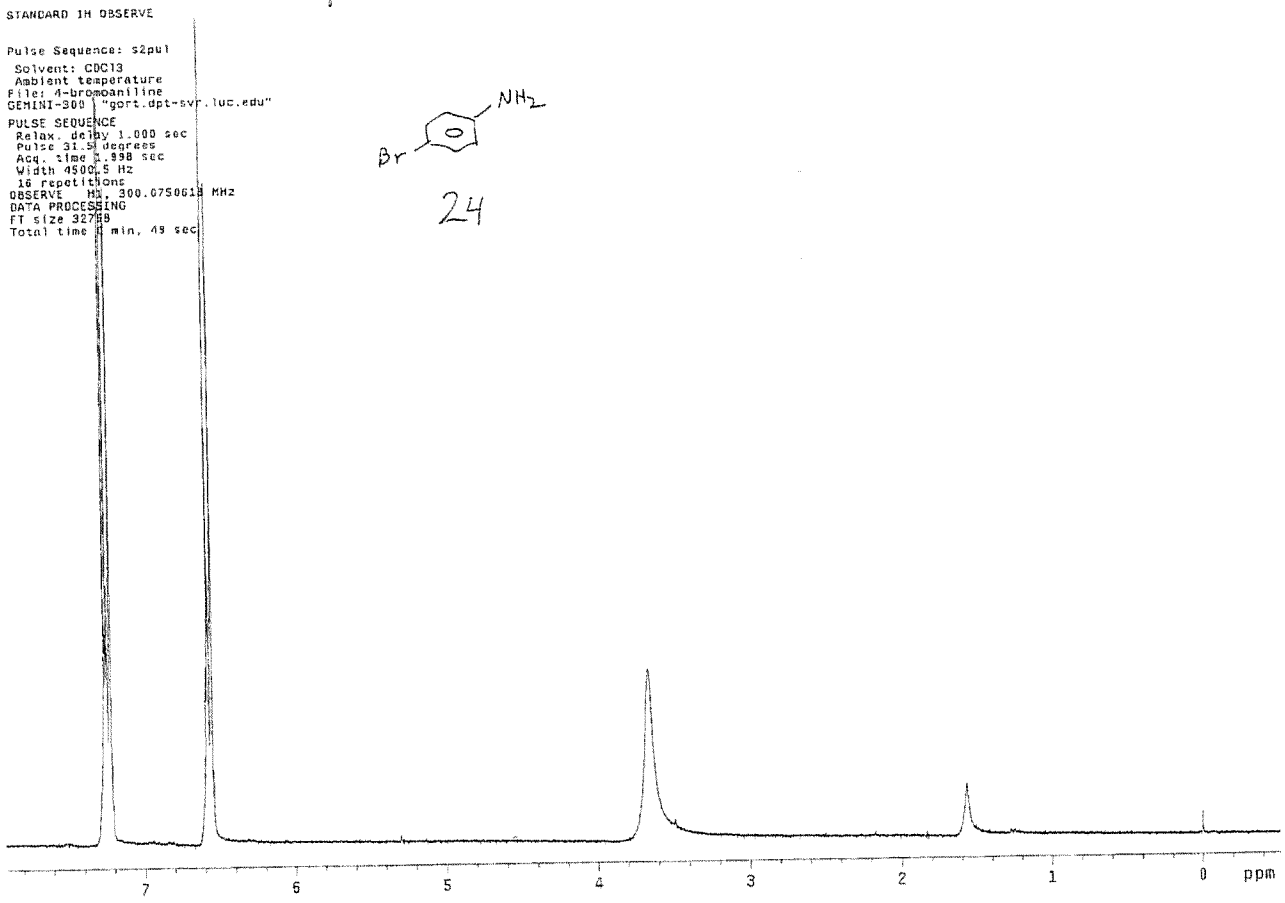
(REAGENT)

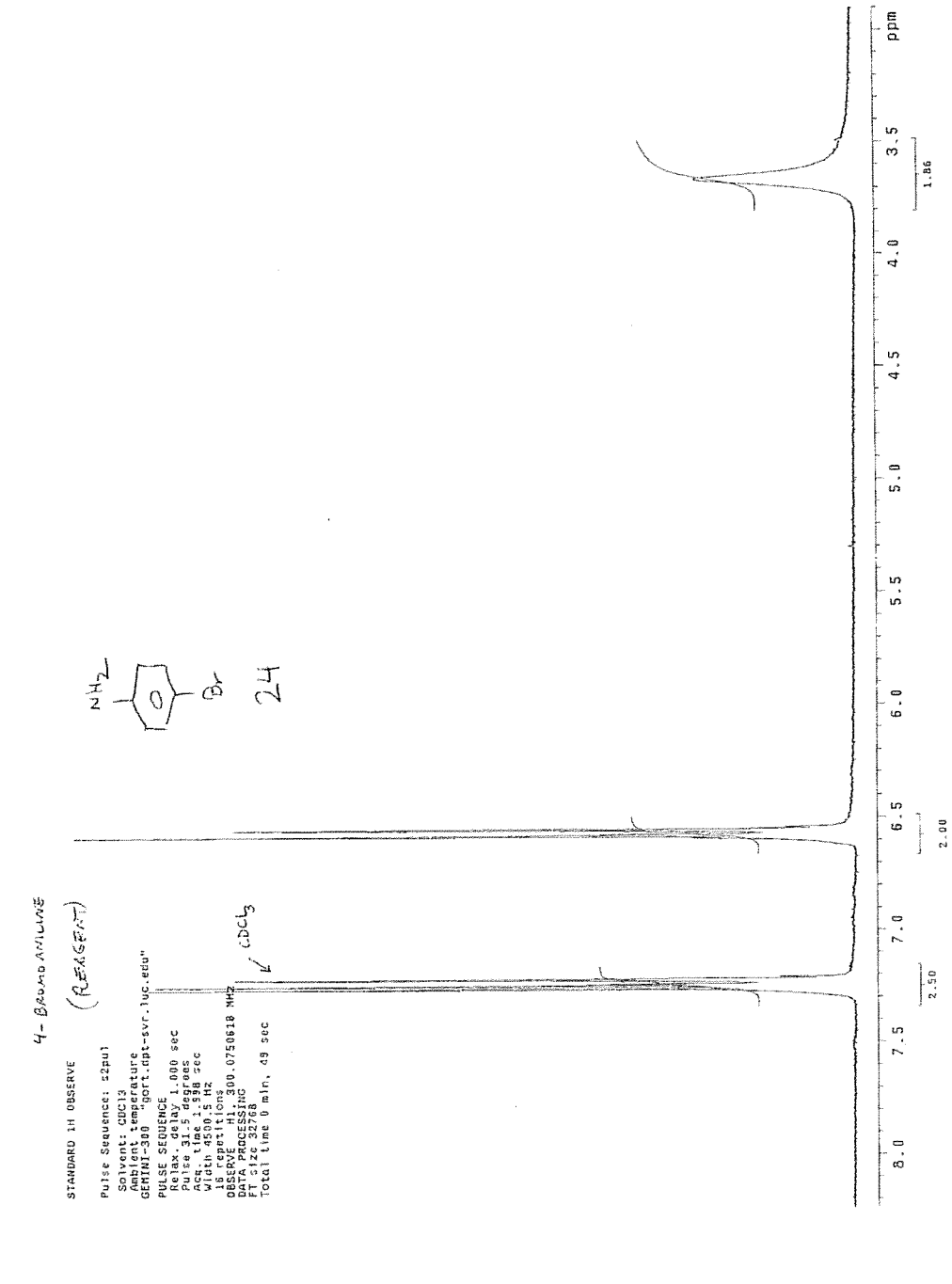
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 31.5 degrees
Acq. time 1.998 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750618 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 49 sec



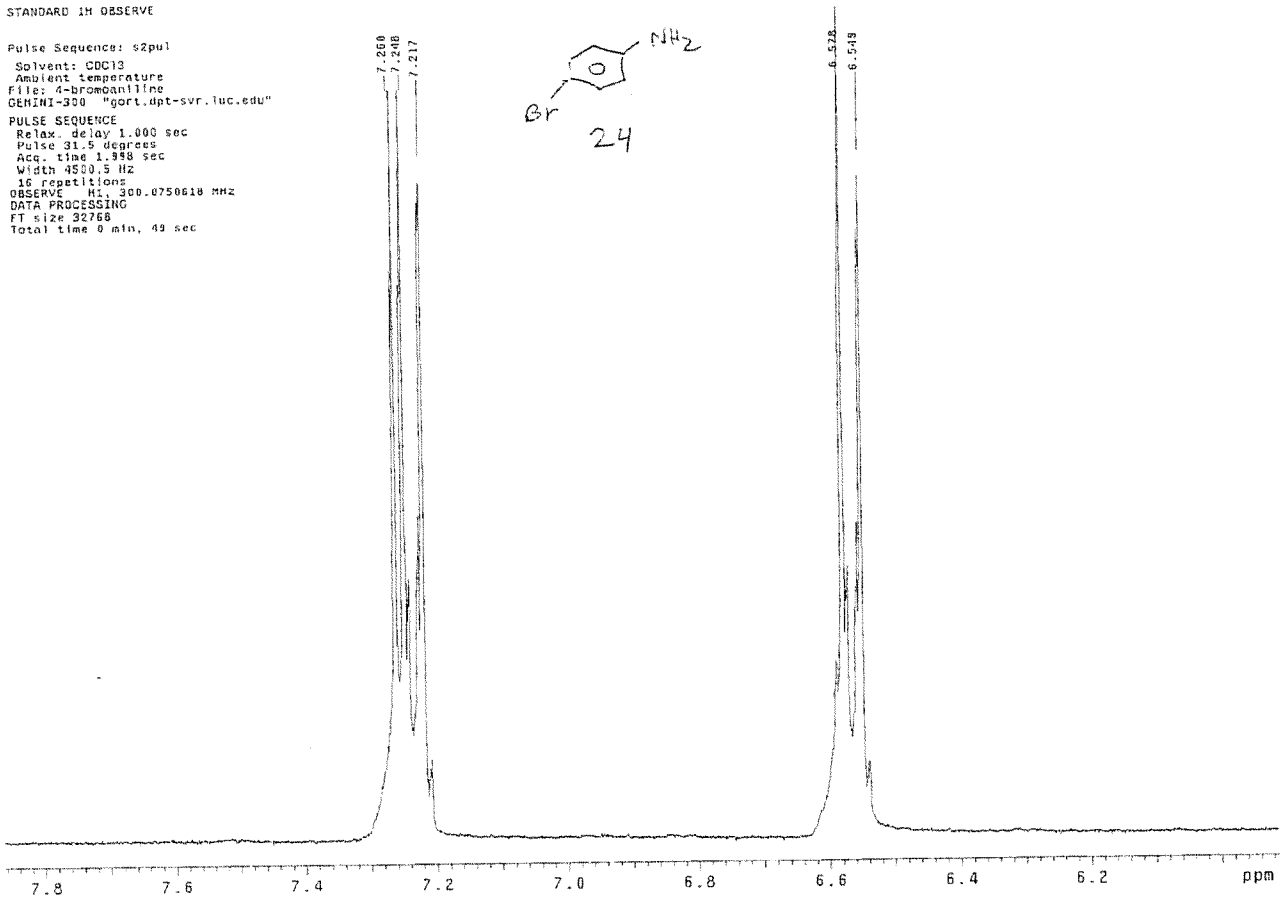
24

4- bromoaniline (reagent)




4- BROMO ANILINE
(REAGENT)
STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 31.5 degrees
Acq. time 1.598 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750618 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 49 sec
NH2
Br
24
CDCl3
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
ppm
2.50
2.00
1.86

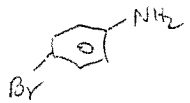
4- BROMO ANILINE (REAGENT)



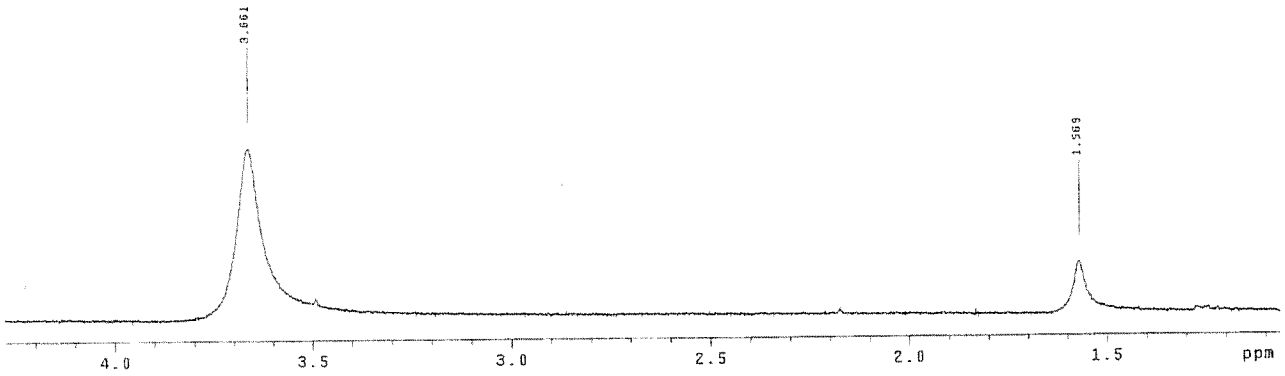
STANDARD 1H OBSERVE

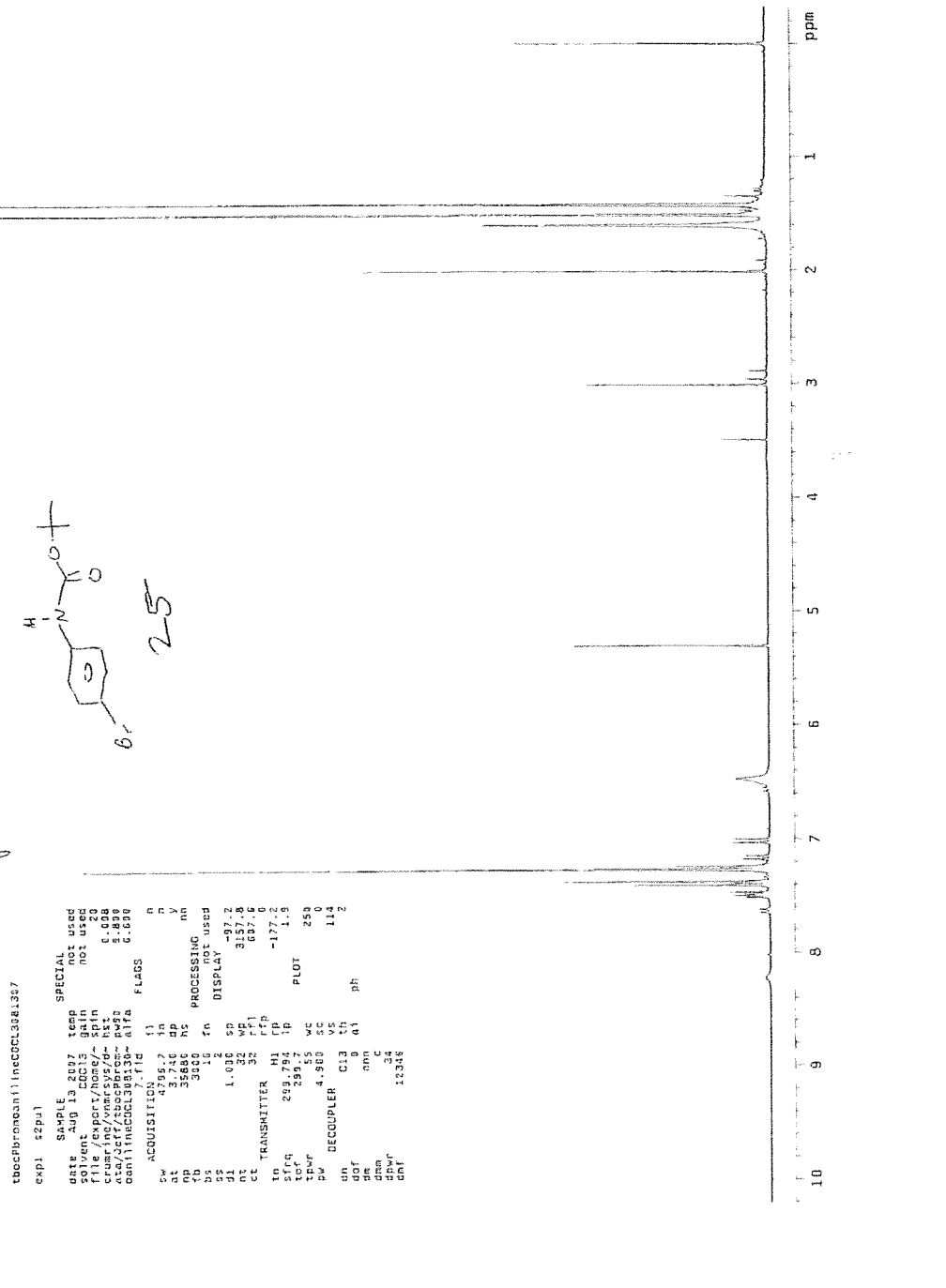
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: 4-bromoaniline
GEMINI-300 "qort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 31.5 degrees
Acq. time 1.998 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750618 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 49 sec

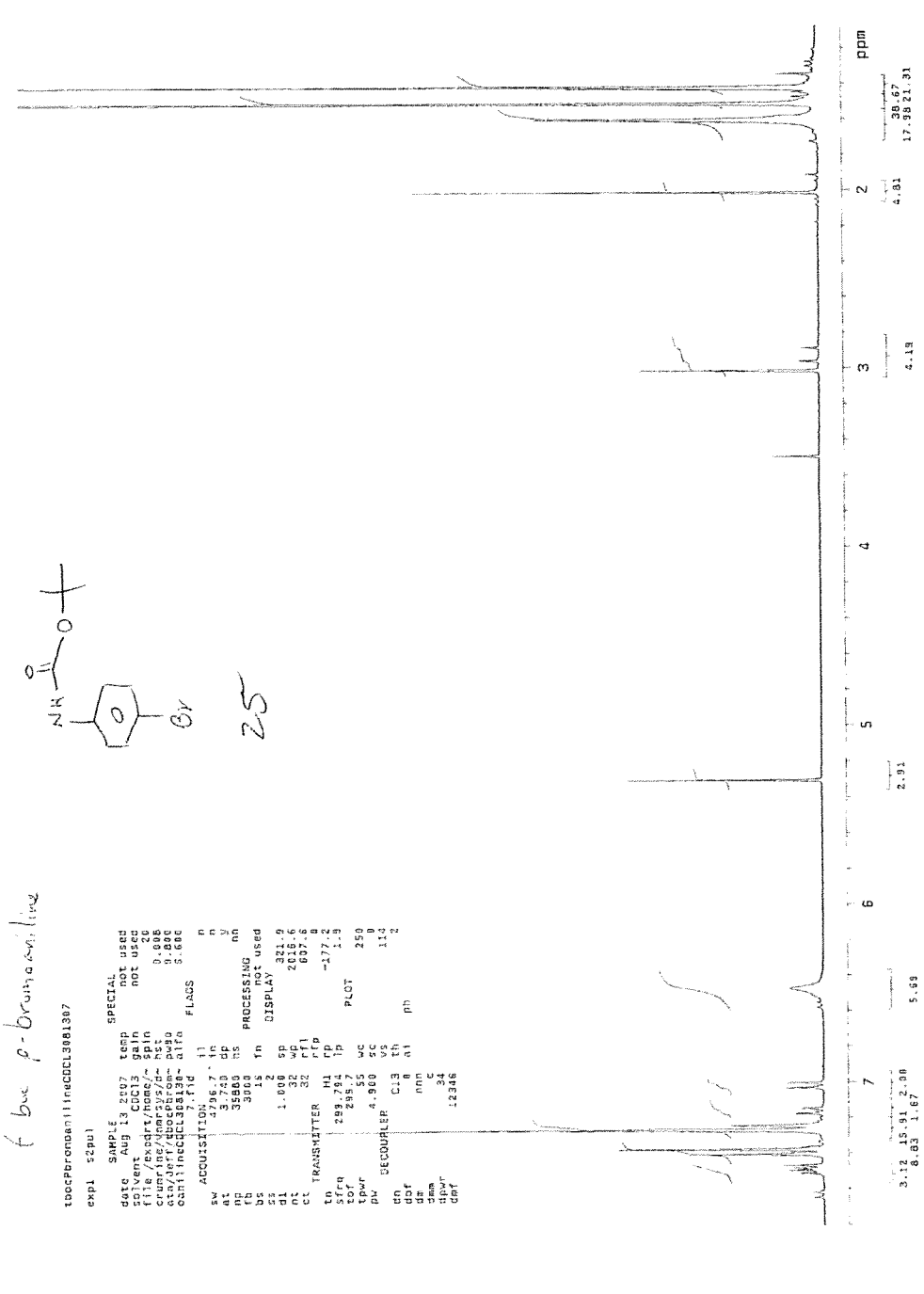
4-BROMOANILINE
(REAGENT)

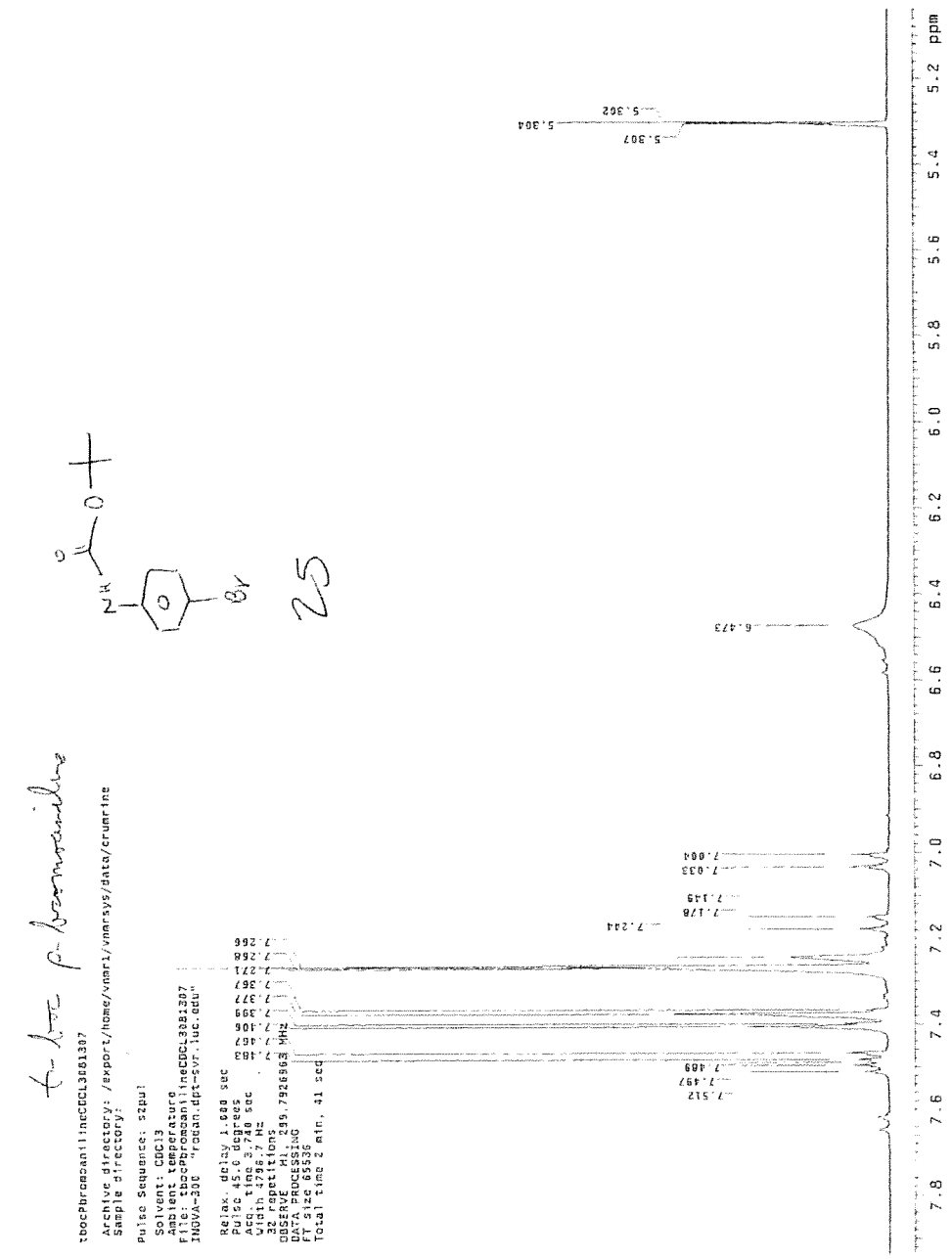


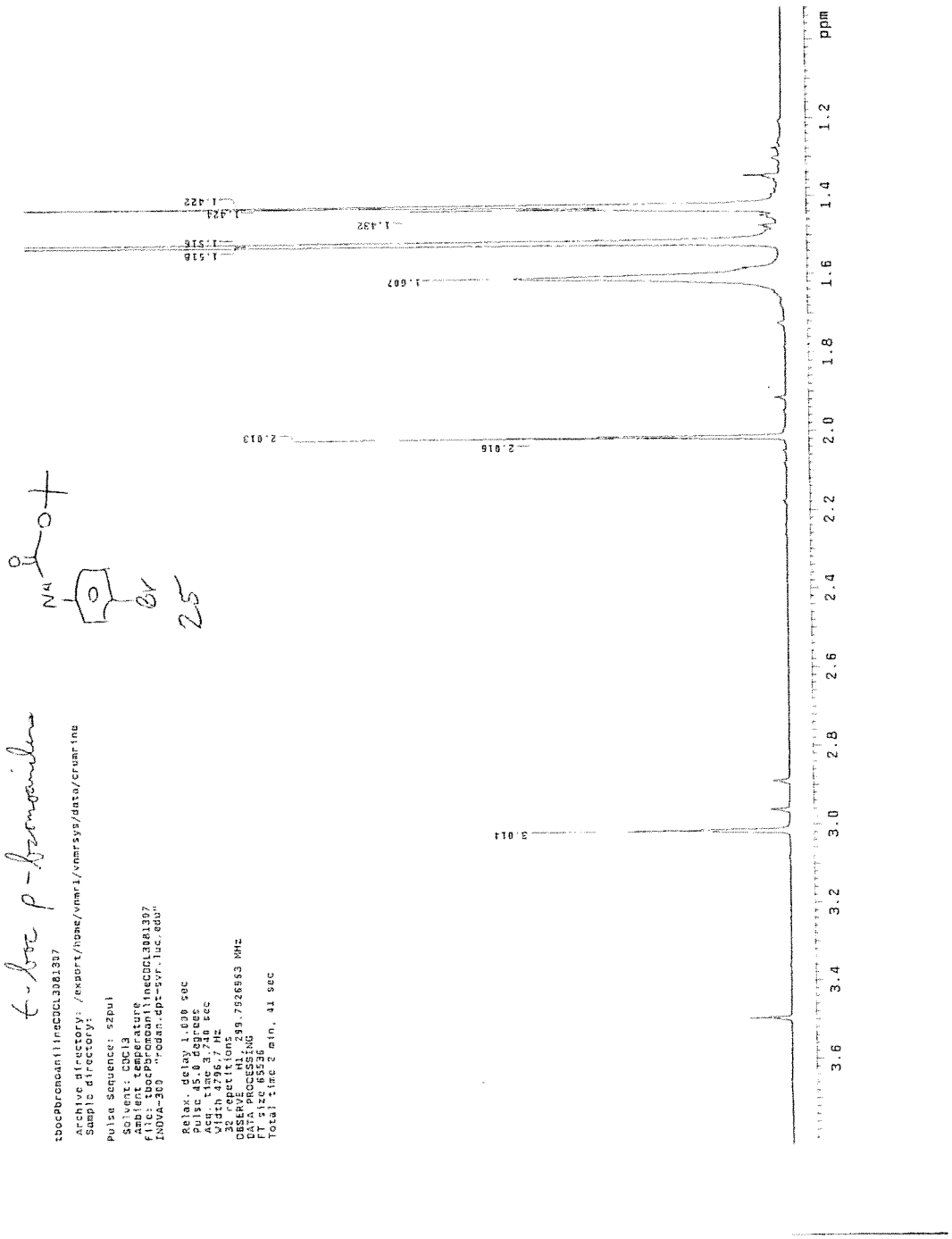
24

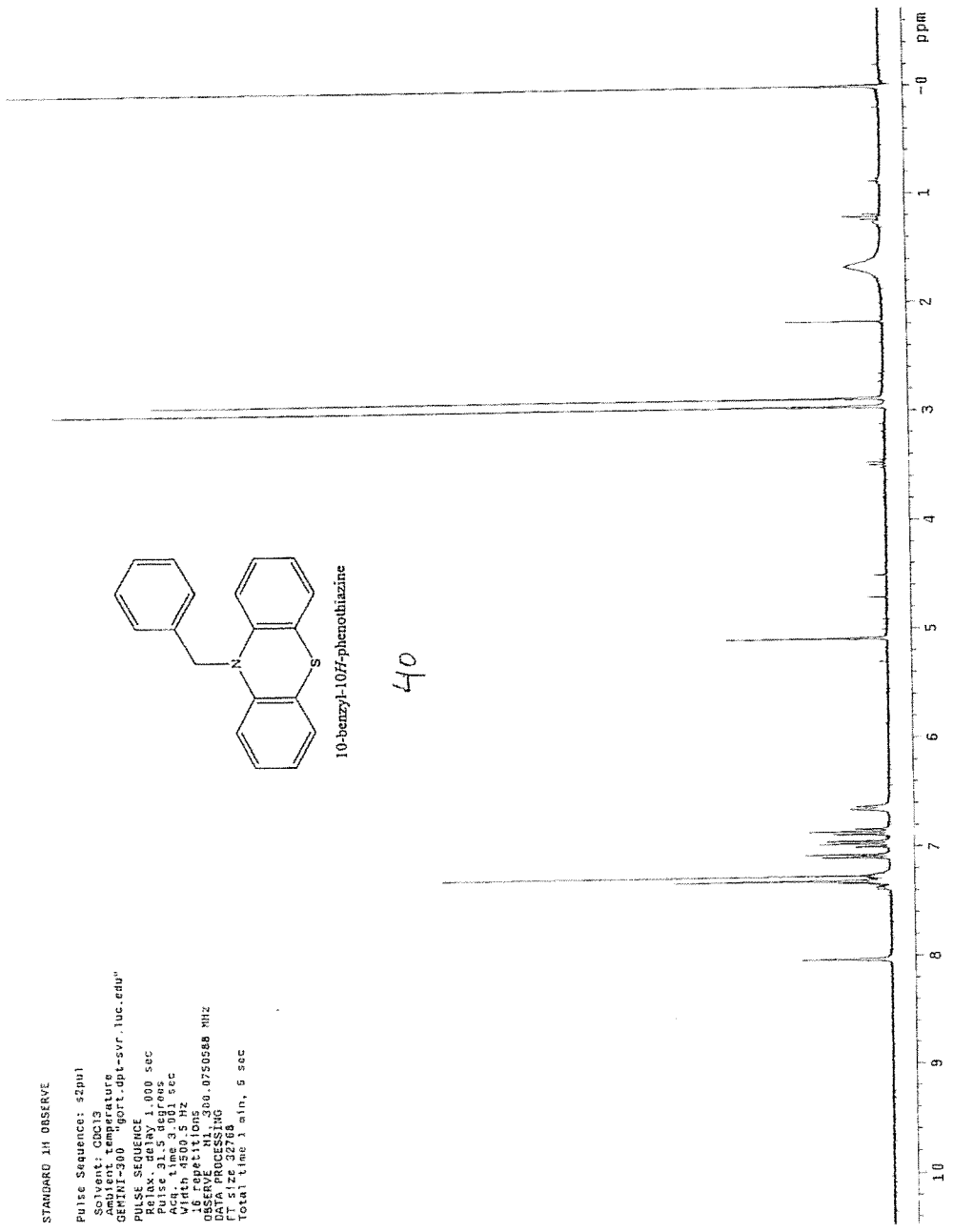


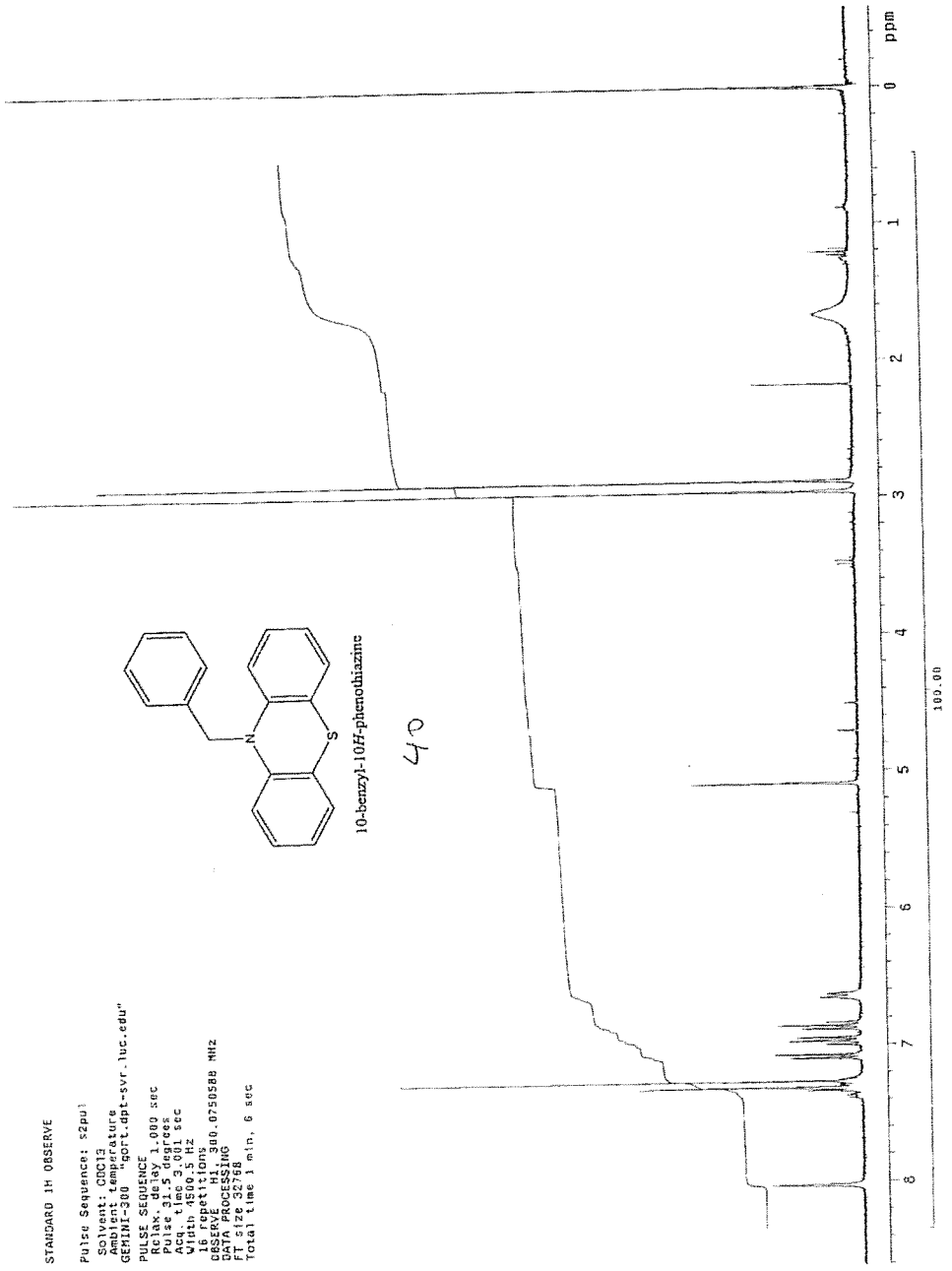

t-boc protected 4-bromoaniline
25
Br
ppm
1
2
3
4
5
6
7
8
9
10


tbocPerondanilineCDCl3081307
expl s2pul
SAMPLE
date Aug 13 2007
solvent cdcl3
file /export/home/jmarcus/d-
crunch /export/home/jmarcus/d-
sta /export/home/jmarcus/d-
sample tbocPerondanilineCDCl3081307
FLAGS
SPECIAL
PROCESSING
DISPLAY
PLOT
DECOUPLER
TRANSMITTER
ppm
3.12 15.91 2.00 5.99
8.83 1.67
2.91
4.19
4.81
38.67
17.98 21.91
Br
25


tbocPbromoanilineCDCL3081307
Archive directory: /export1/home/vnmrj/vnmrsys/data/crumrine
Sample directory:
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: tbocPbromoanilineCDCL3081307
INOVA-300 "rogan.dpt-svr.luc.edu"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 3.740 sec
Width 4796.7 Hz
32 repetitions
OBSERVE H1, 299.7926908 MHz
DATA PROCESSING
FT size 65536
Total time 2 min, 41 sec
7.512
7.497
7.489
7.463
7.462
7.183
7.106
7.399
7.377
7.367
7.221
7.268
7.266
7.244
7.178
7.149
7.033
7.004
6.473
5.307
5.304
5.302
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
5.2
ppm

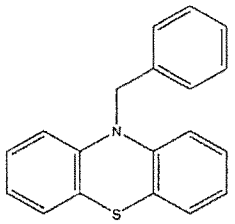

t-Boc p-Bromoaniline
tbocPbromoaniline
CDCl3
Archive directory: /export/home/vnmrj/vnmrsys/data/cruarline
Sample directory:
Pulse sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: tbocPbromoaniline
INOVA-300 "rodan.dpz-gvr.luc.edu"
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 3.740 sec
Width 4796.7 Hz
32 repetitions
OBSERVE H1, 299.7526563 MHz
DATA PROCESSING
FT size 65536
Total time 2 min, 41 sec
NH
Br
25
3.014
2.016
2.013
1.607
1.516
1.432
1.424
1.422
3.6
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
ppm


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 30.5 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750588 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
10-benzyl-10H-phenothiazine
40
ppm


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 45.0 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750588 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
10-benzyl-10H-phenothiazine
40
N
S
ppm
0
1
2
3
4
5
6
7
8
100.00

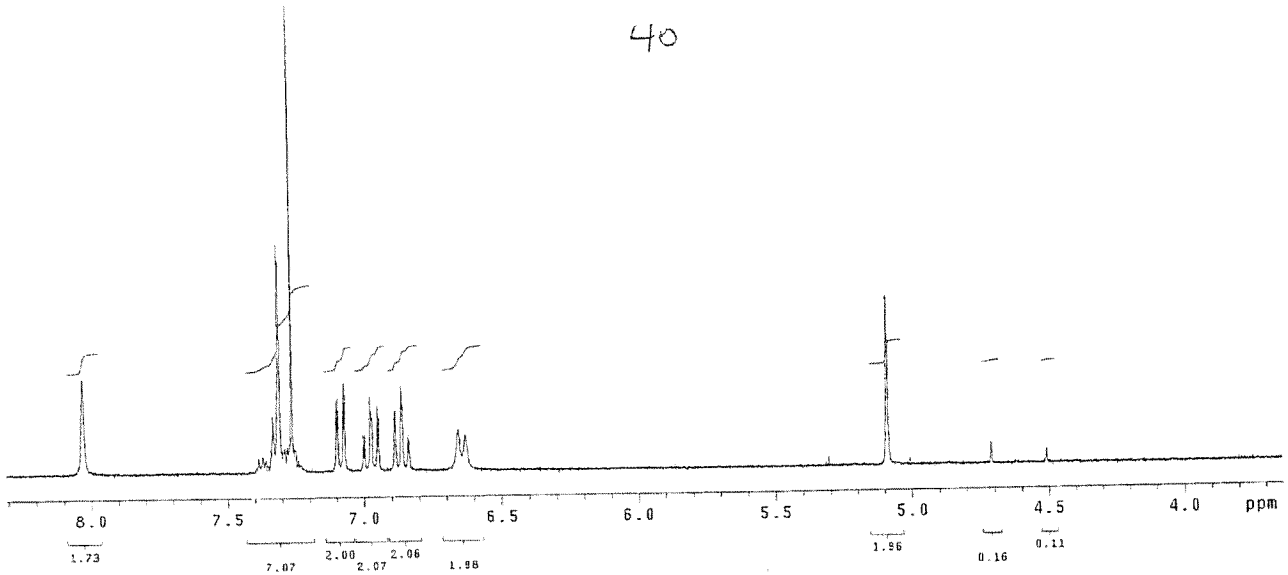
STANDARD 1H OBSERVE

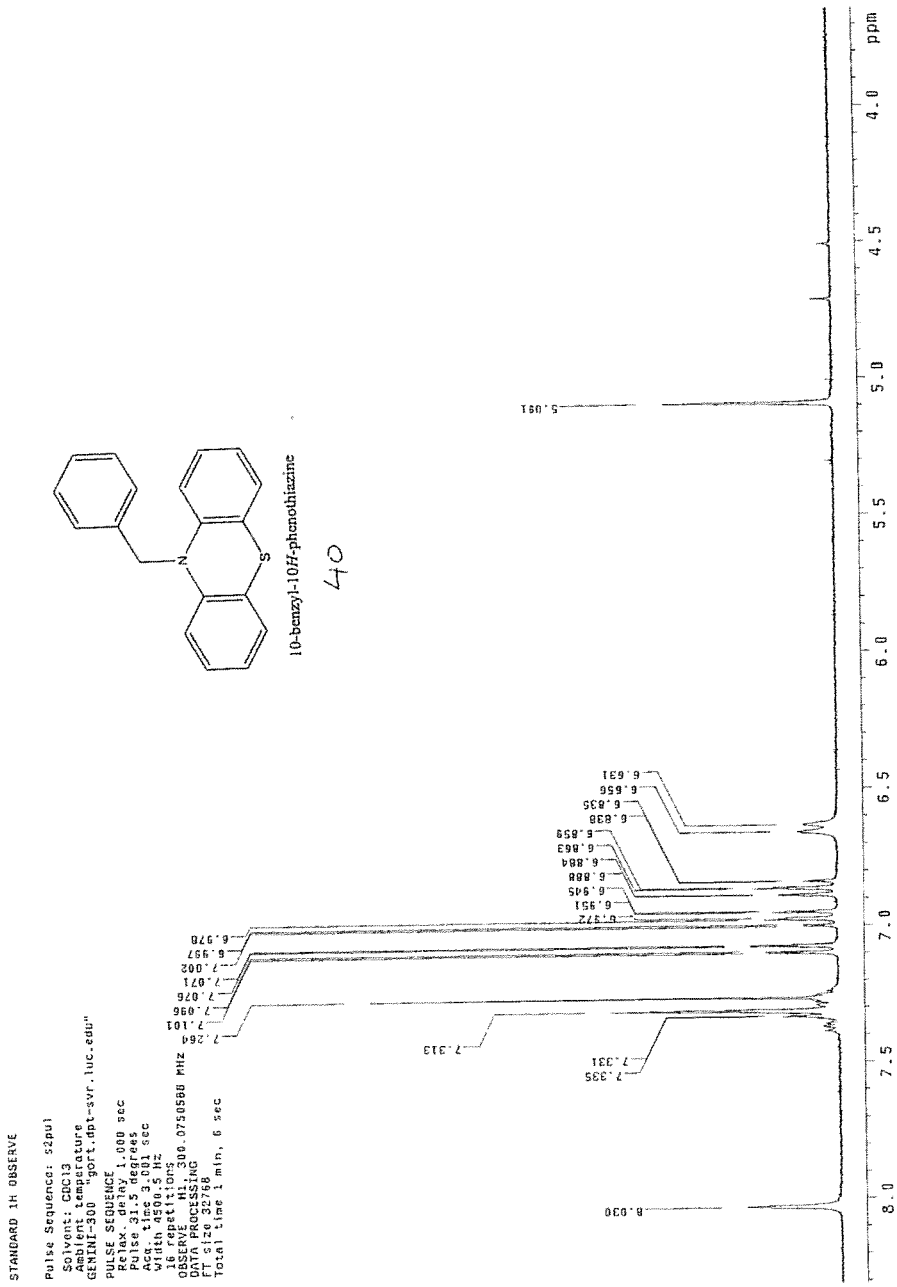
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 31.5 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750588 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec

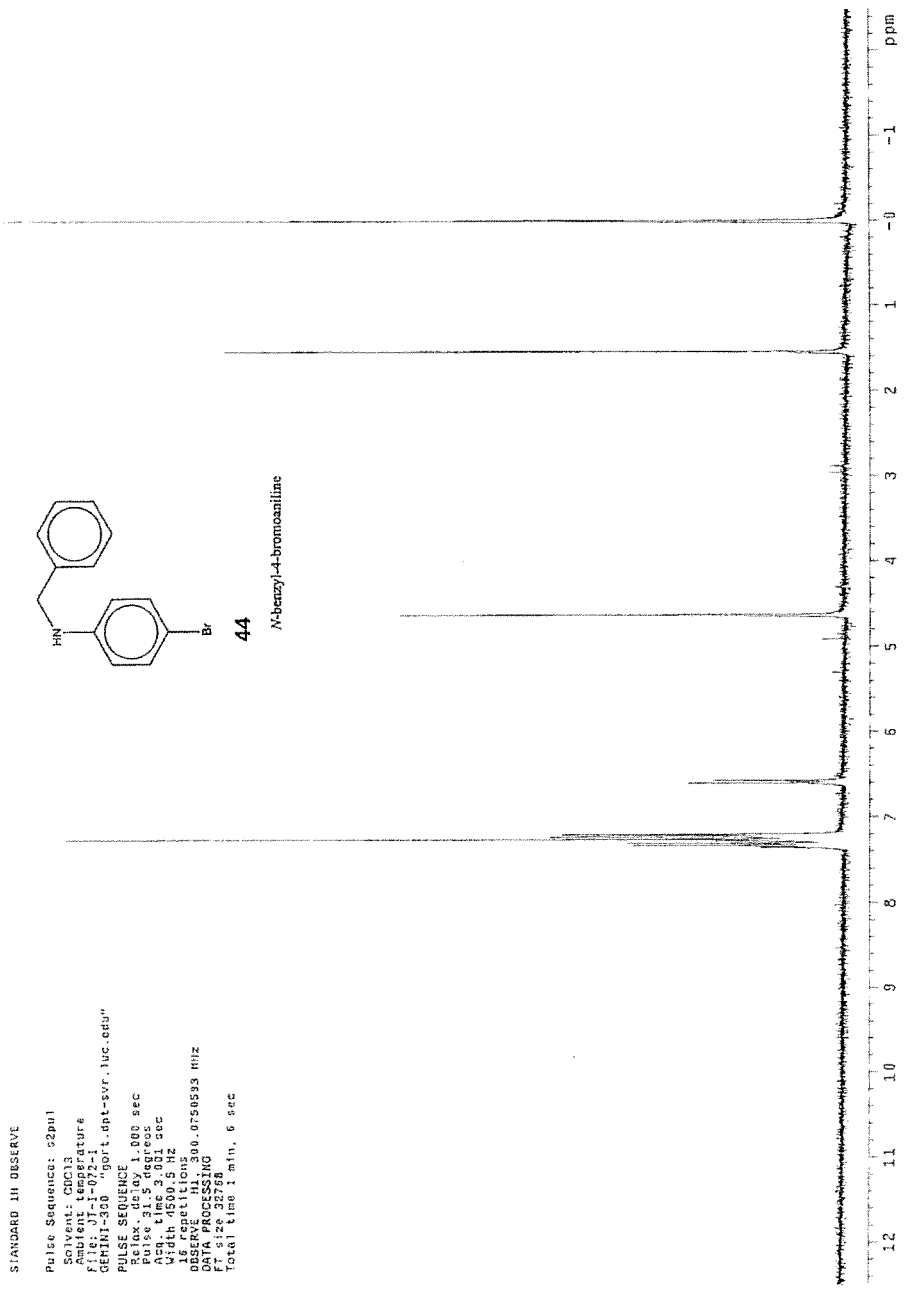


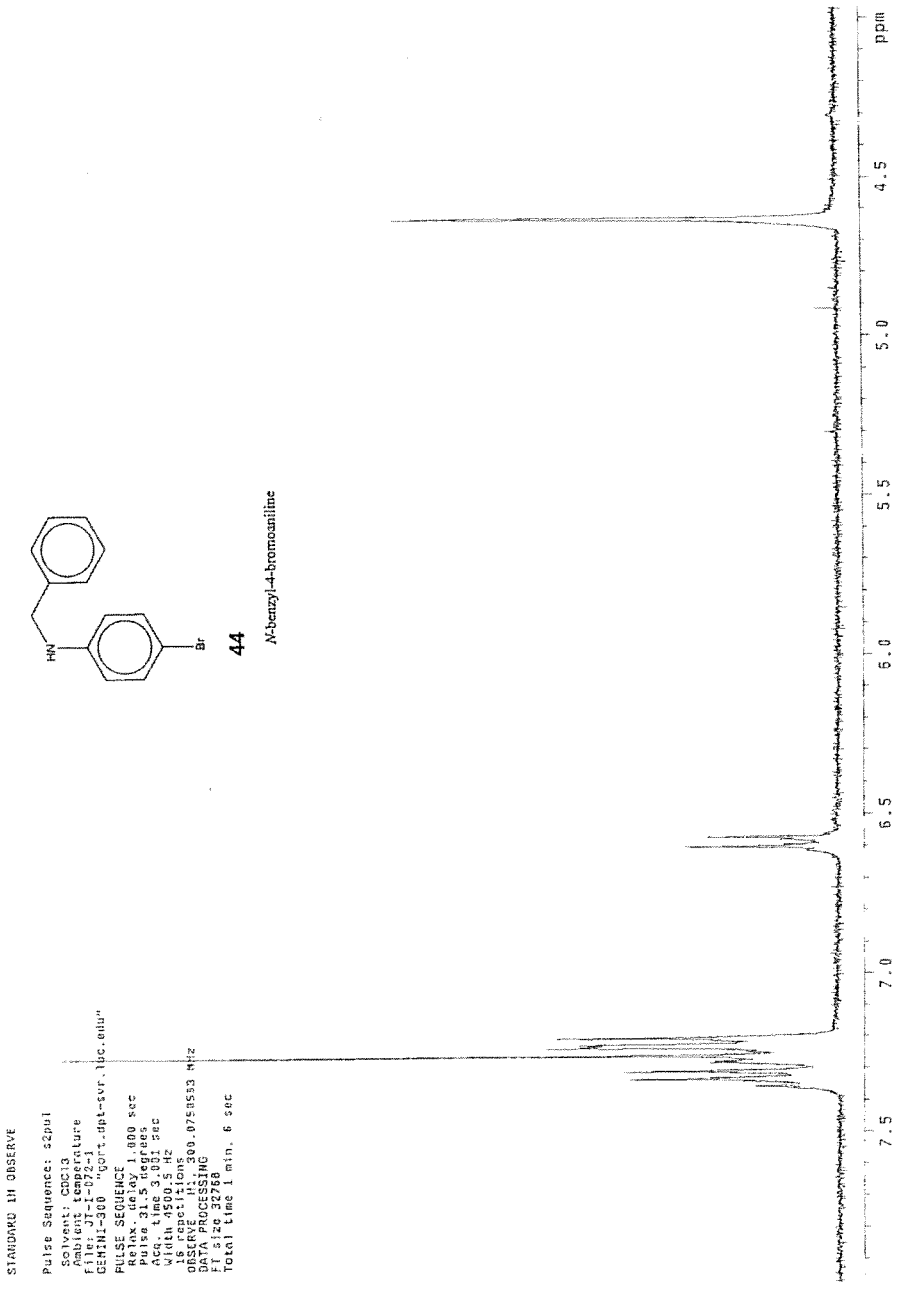
10-benzyl-10*H*-phenothiazine

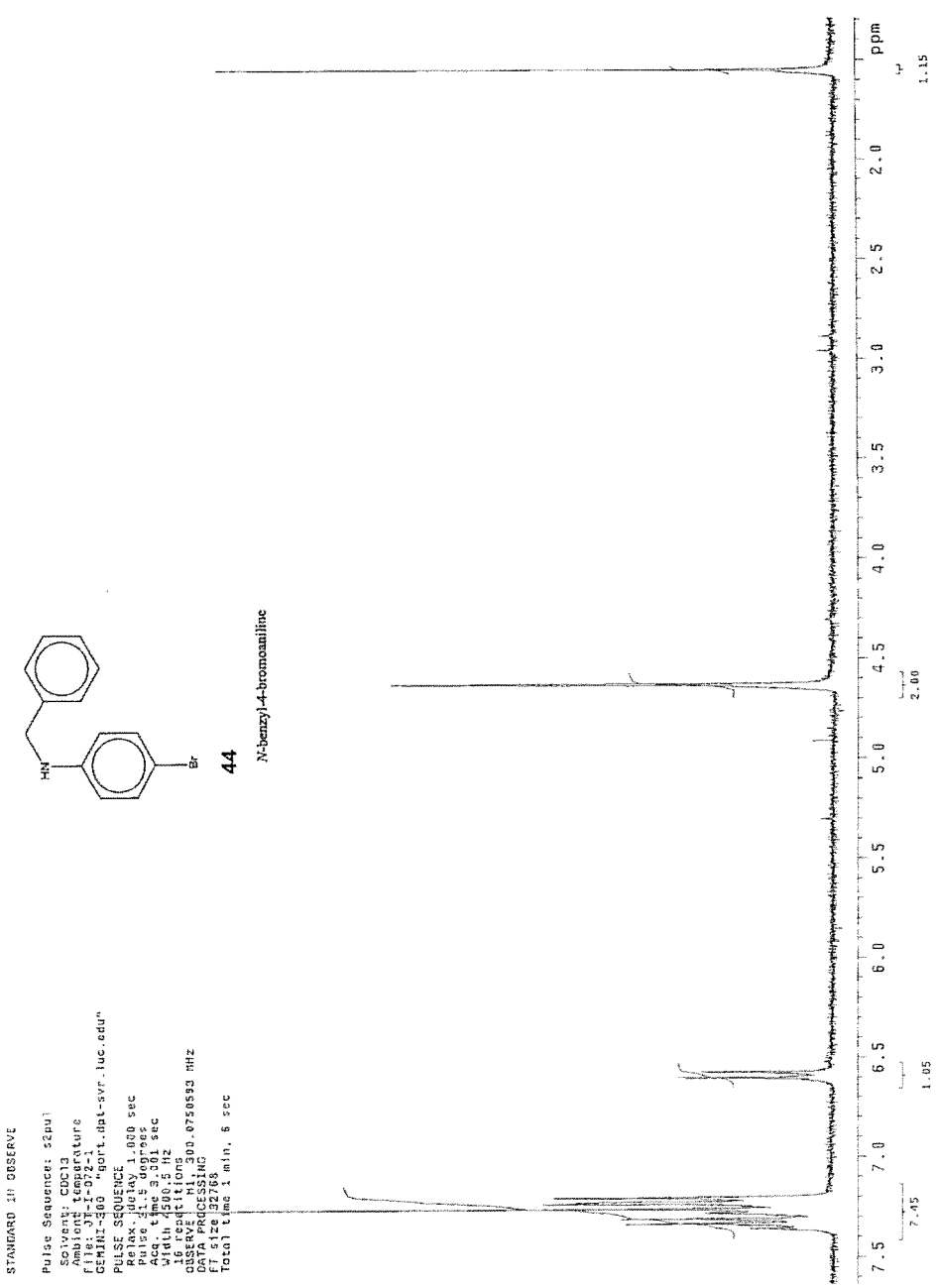
40

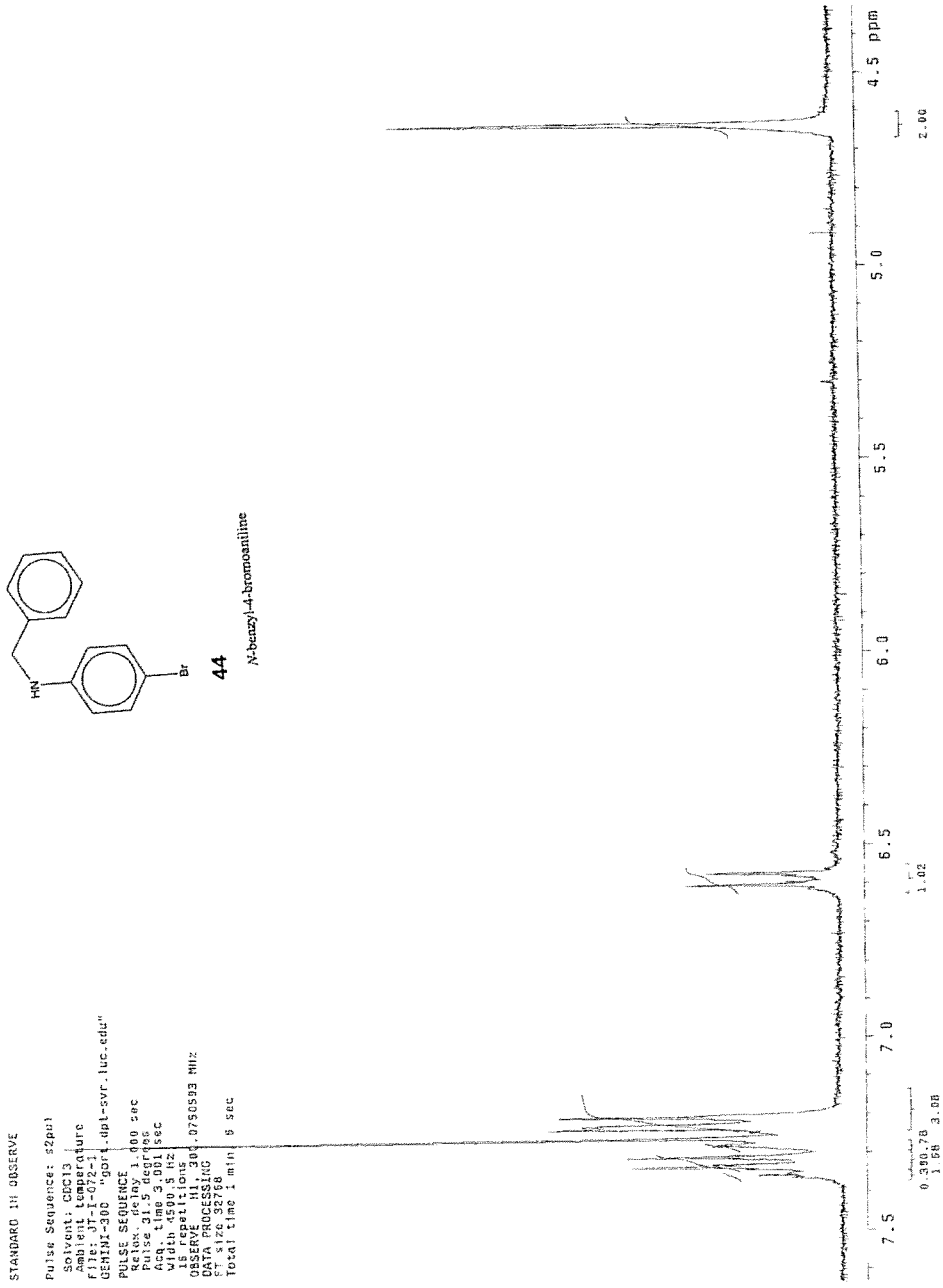


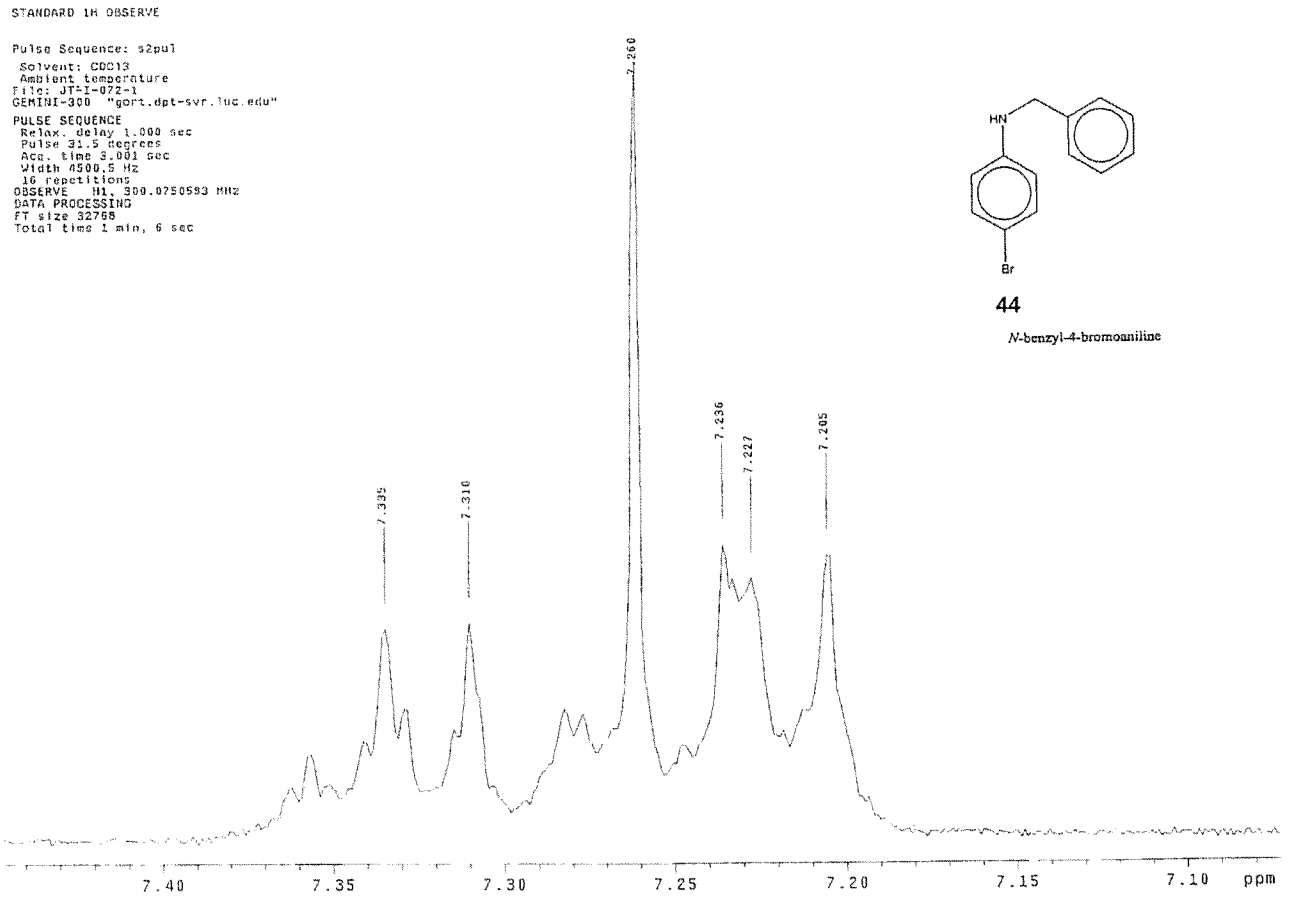

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 30.3 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750580 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
10-benzyl-10H-phenothiazine
40
N
S
5.091
8.030
7.313
7.335
7.331
7.264
7.101
7.096
7.076
7.071
7.002
6.997
6.970
6.972
6.951
6.945
6.888
6.884
6.863
6.859
6.838
6.835
6.656
6.631
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
ppm


SIANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: JT-I-072-1
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 31.5 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750593 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
HN
Br
44
N-benzyl-4-bromoaniline
12
11
10
9
8
7
6
5
4
3
2
1
-0
-1
ppm


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: JT-1-072-1
GEMINI-300 "gsrs.dpt-svr.ibc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 31.5 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0758553 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
HN
Br
44
N-benzyl-4-bromoaniline
7.5
7.0
6.5
6.0
5.5
5.0
4.5
ppm


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: JT-I-072-1
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 31.5 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750593 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
HN
Br
44
N-benzyl-4-bromoaniline
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm
7.45
1.05
2.00
1.15


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: JT-I-072-1
GEMINI-300 "gort.dpt-cvr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 31.5 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750593 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 5 sec
HN
Br
44
N-benzyl-4-bromoaniline
7.5
7.0
6.5
6.0
5.5
5.0
4.5 ppm
0.390.78
1.68
3.08
1.02
2.00

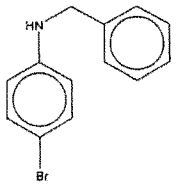


44

N-benzyl-4-bromoaniline

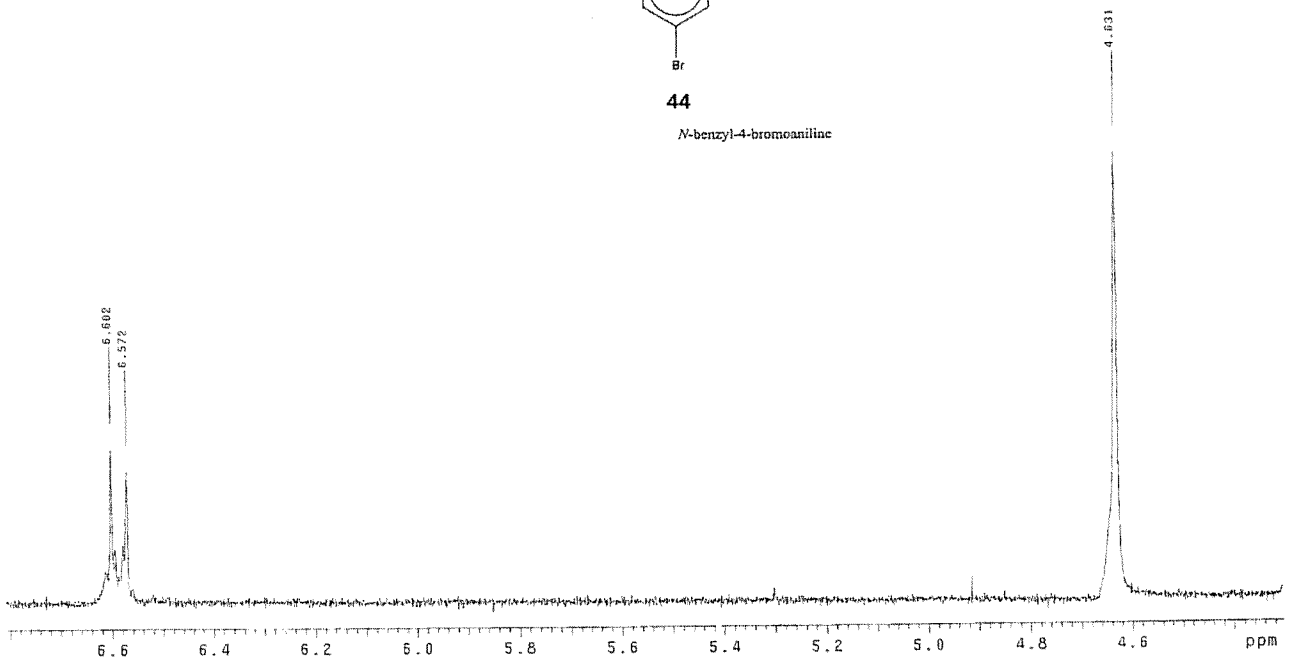
STANDARD 1H OBSERVE

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: JT-I-072-1
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 31.5 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750593 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec



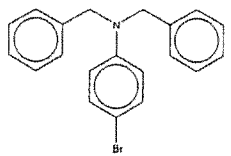
44

N-benzyl-4-bromoaniline



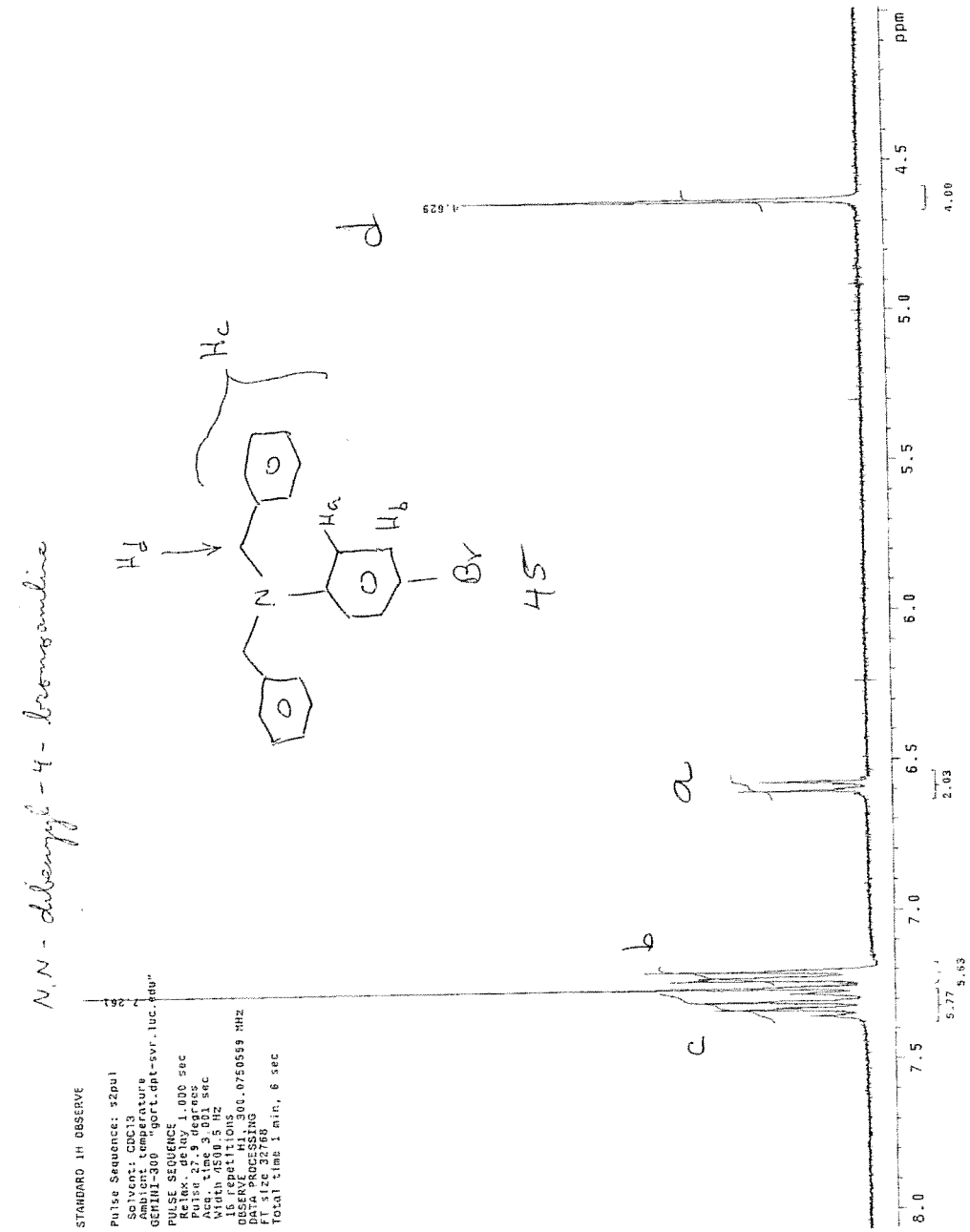
STANDARD 1H OBSERVE

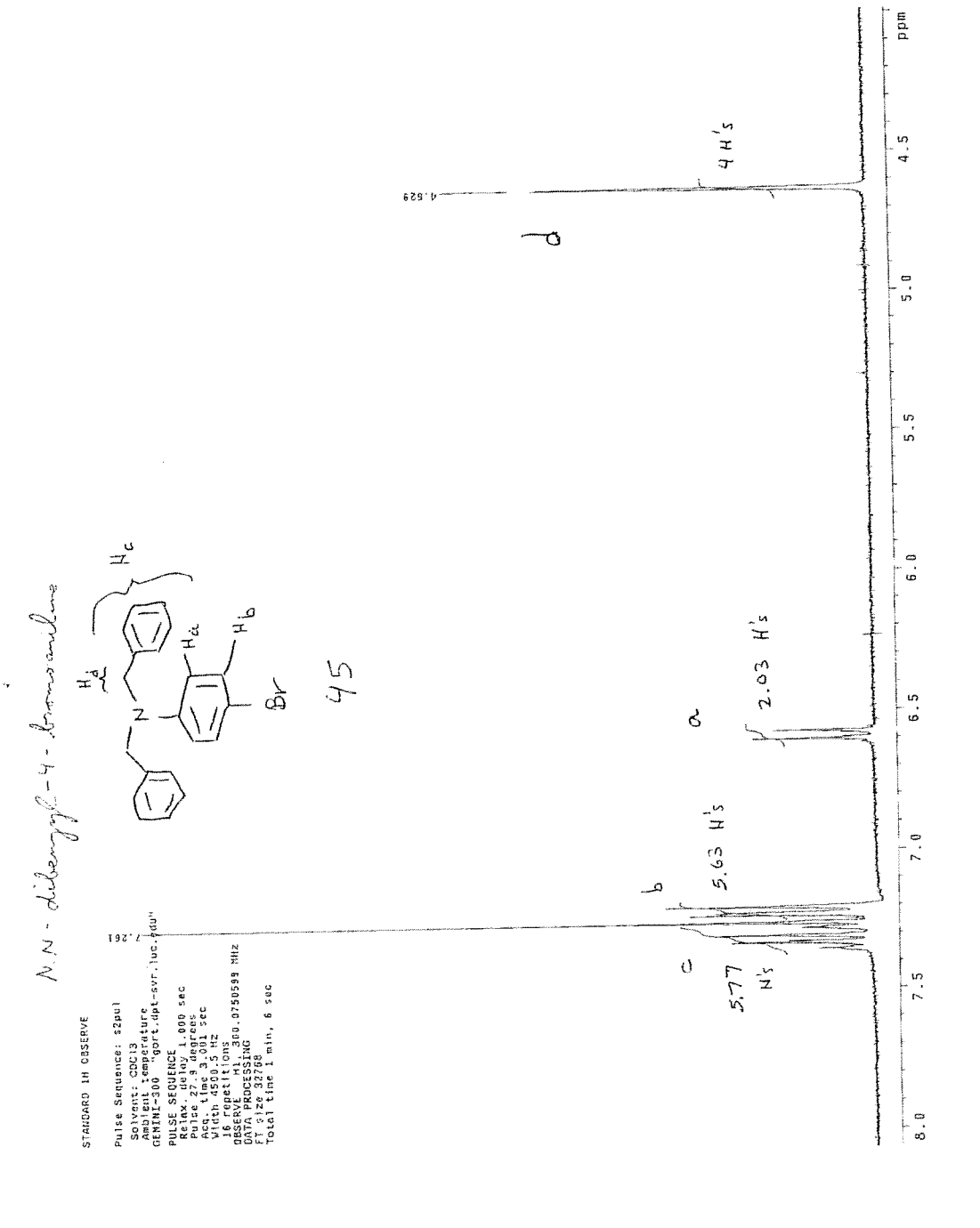
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750599 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec



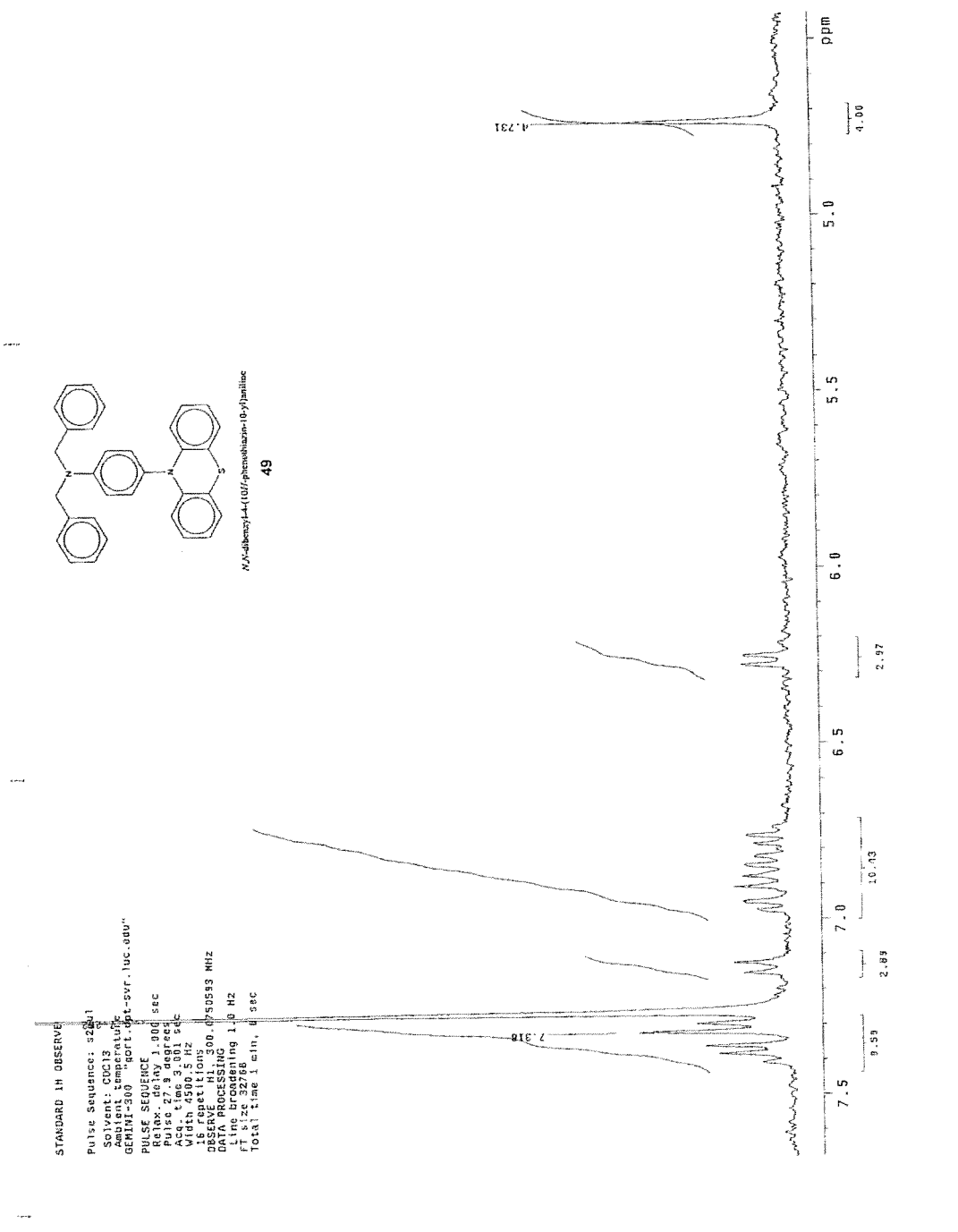
45

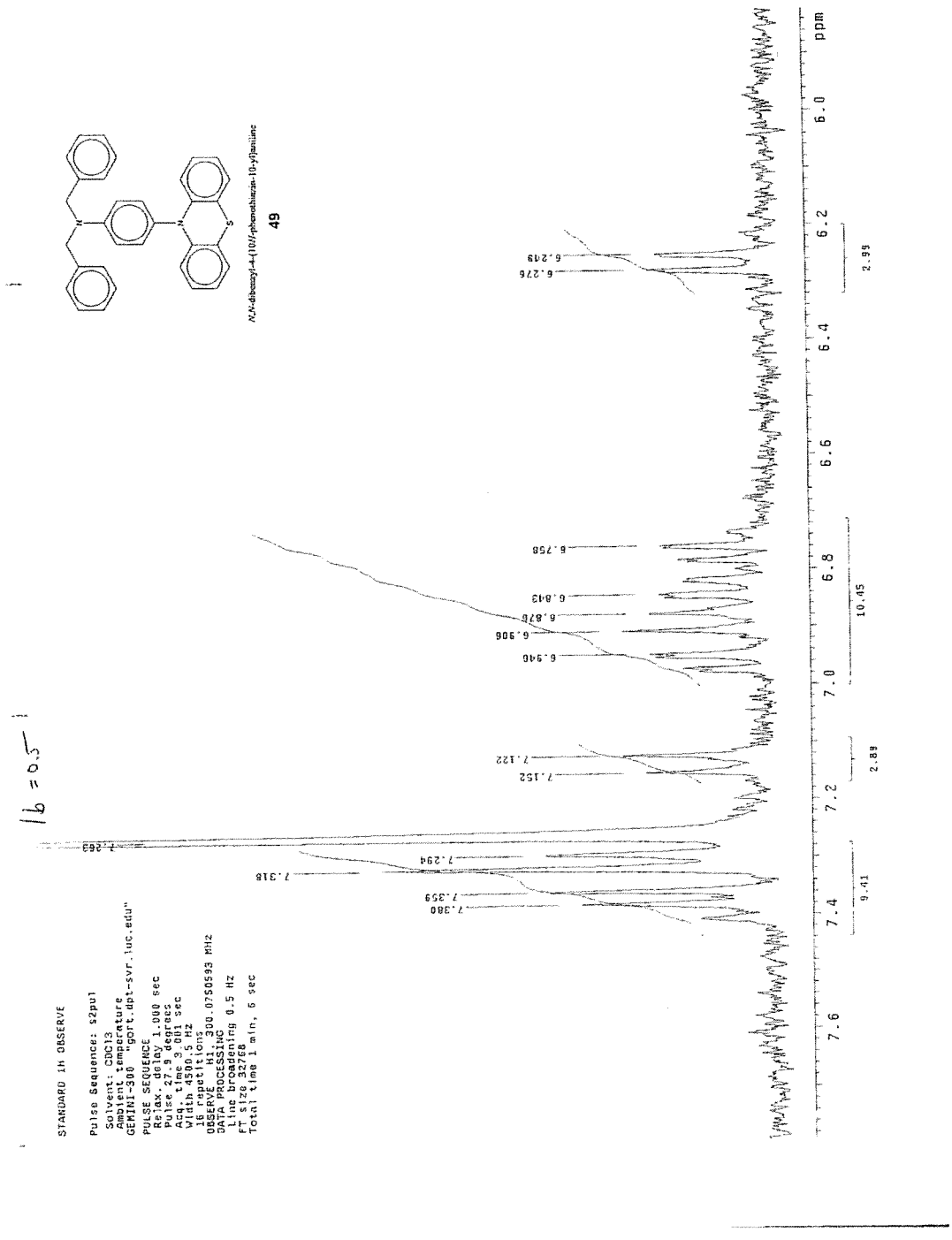
N,N-dibenzyl-4-bromoaniline

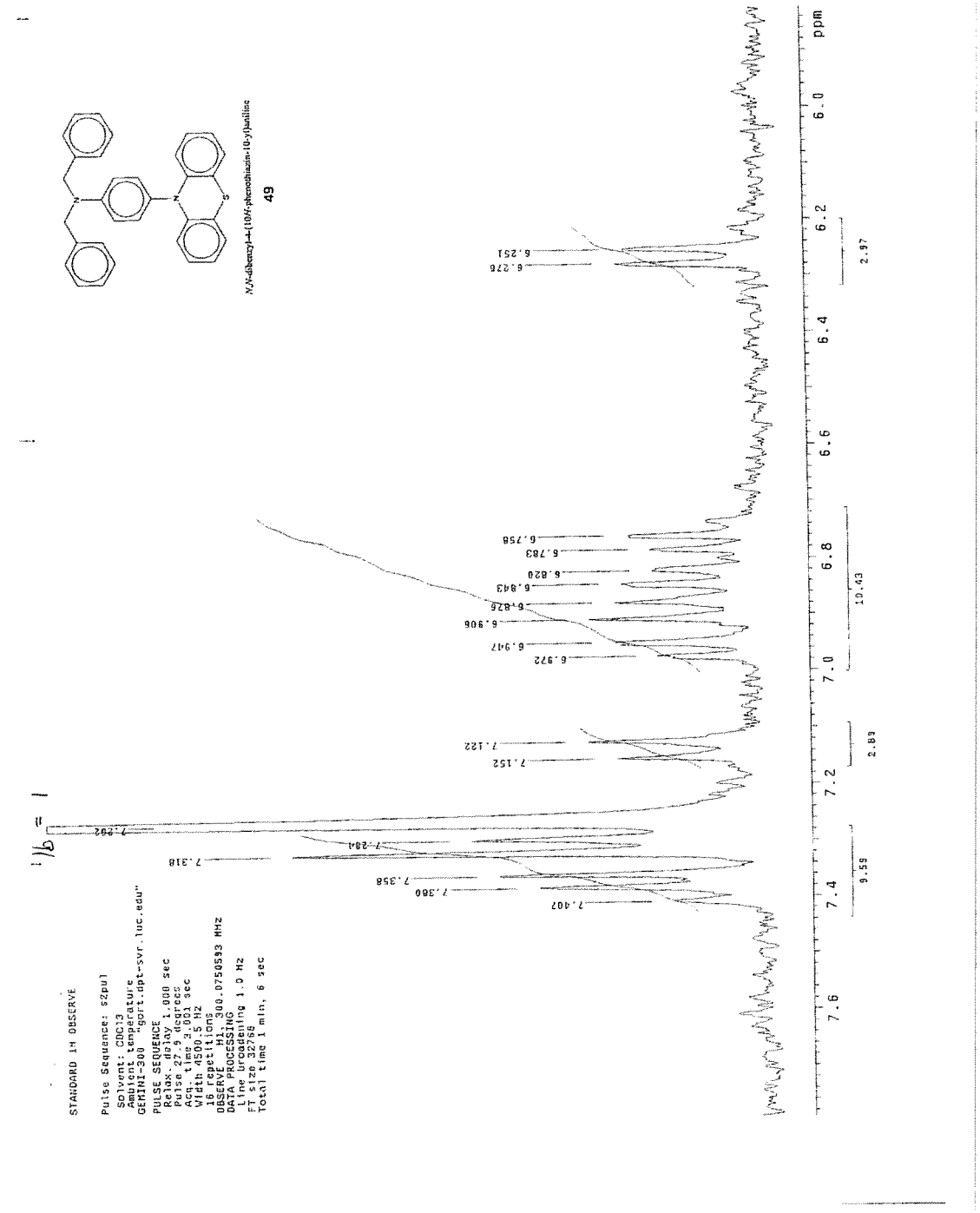

N,N - dibenzyl - 4 - bromoaniline
STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
RELAX. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750599 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
Ha
Hb
Hc
Hd
Br
45
a
b
c
d
4.629
5.77
5.63
2.03
4.00
ppm

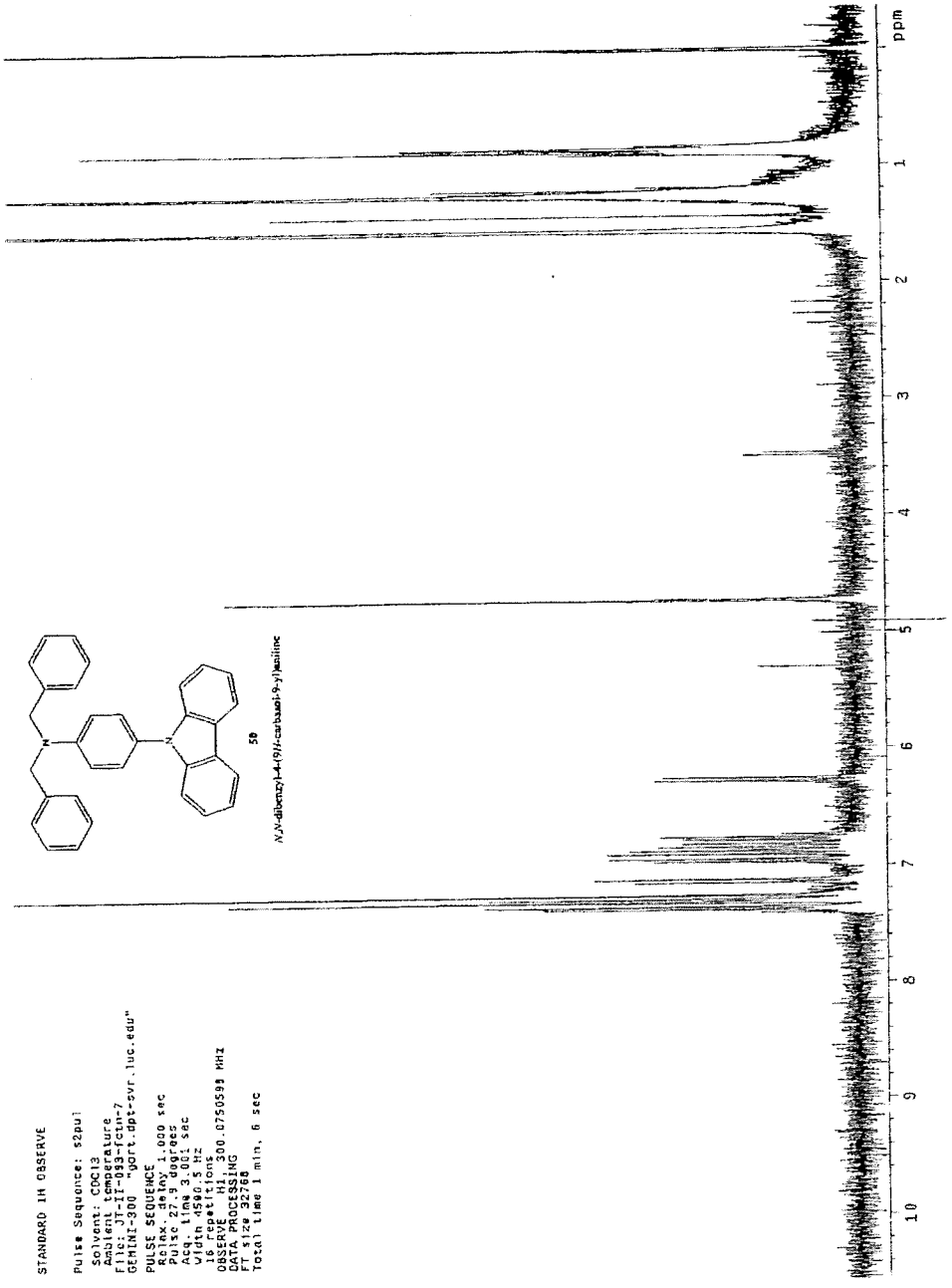

N,N - dibenzyl - 4 - bromoaniline
45
STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 'gort.4pt-svr.luc.pdu'
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.4 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750599 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
4 H's
2.03 H's
5.63 H's
5.77
N's
ppm

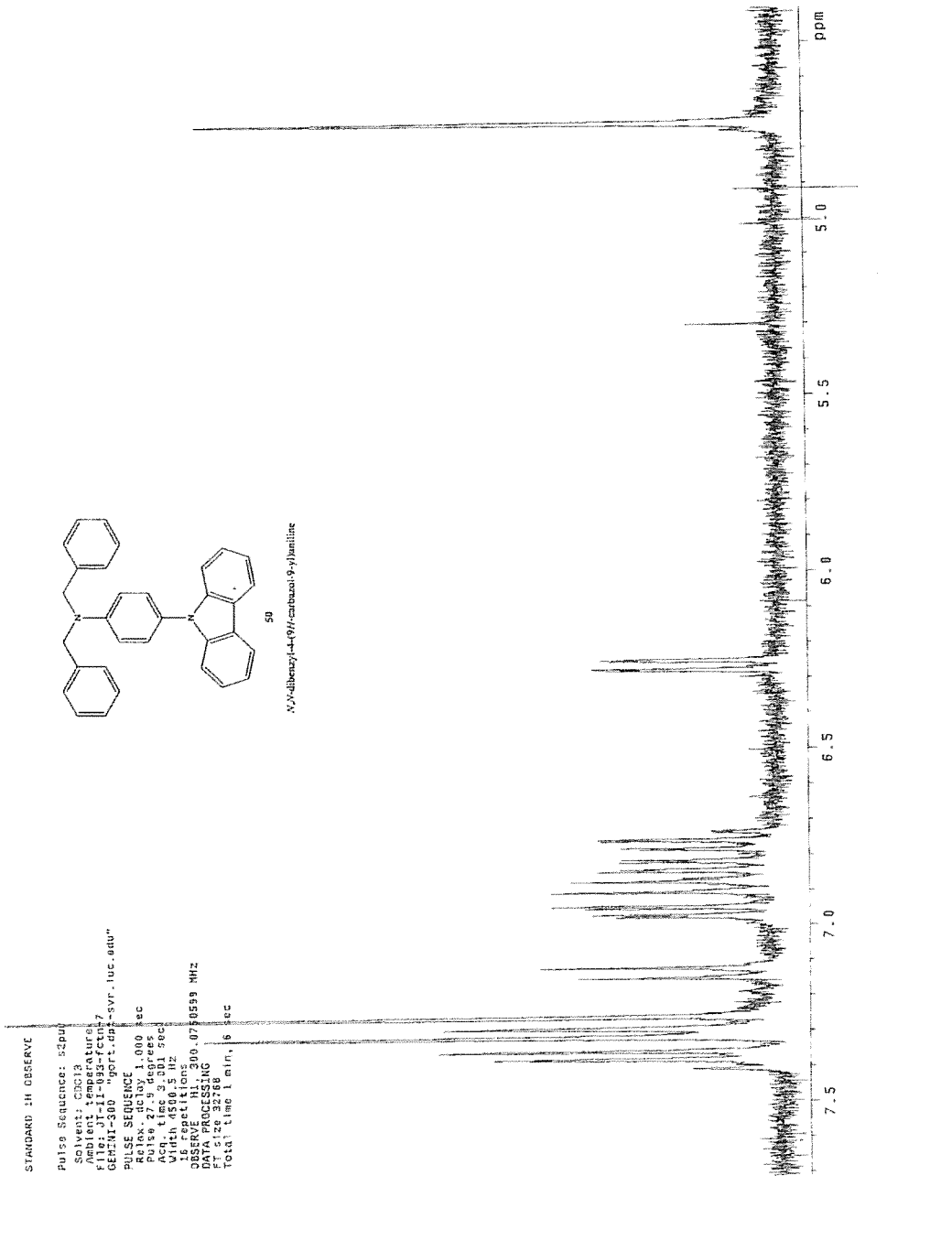

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "pgort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.3 degrees
Acq. time 3.001 sec
Width 45015 Hz
16 repetitions
OBSERVE H1, 300.0759583 MHz
DATA PROCESSING
Line broadening 1.0 Hz
FT size 32768
Total time 1 min, 6 sec
49
N,N-dibenzyl-4-(10H-phenothiazin-10-yl)aniline
ppm

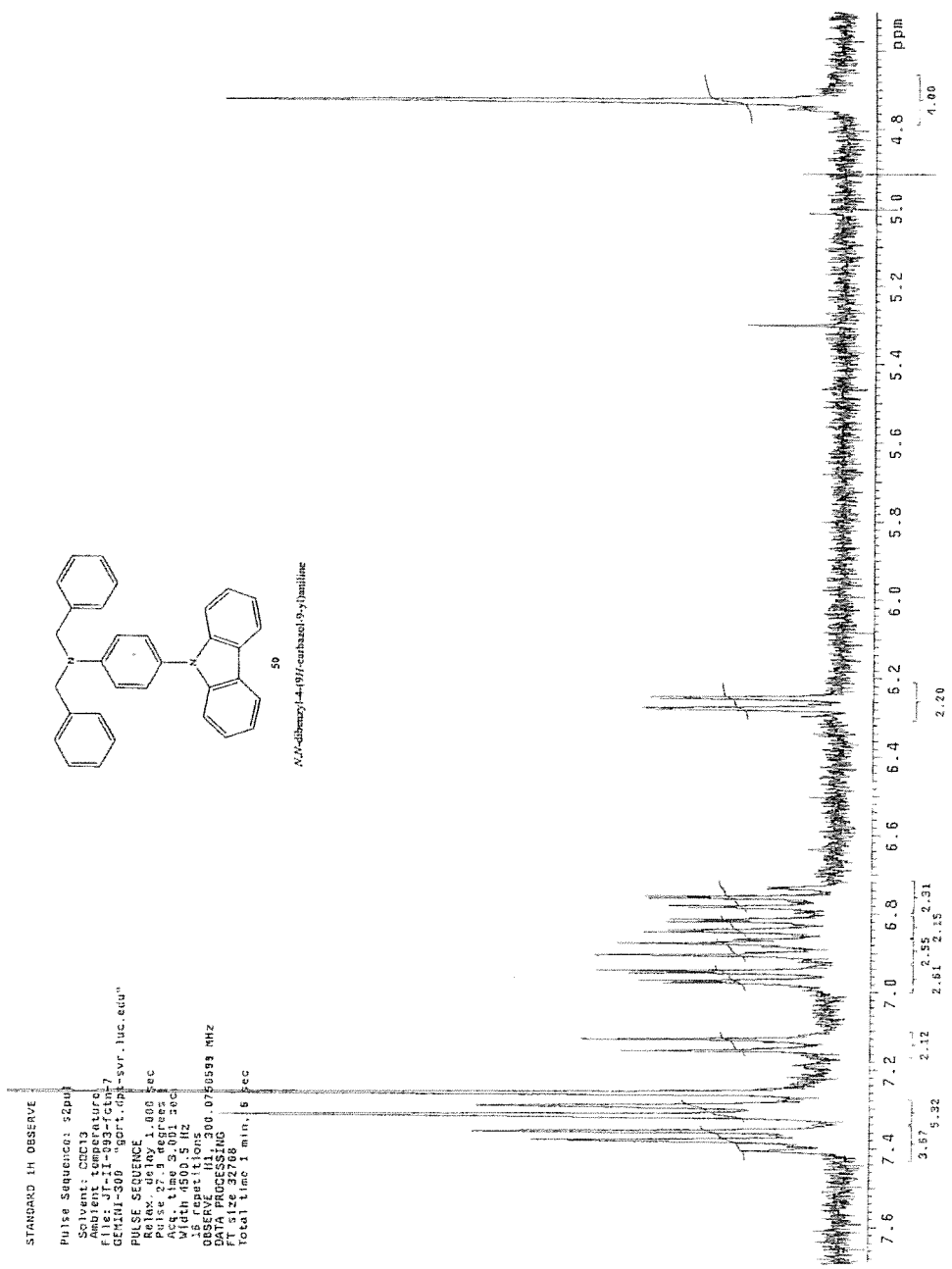

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "qori.dat-svr.luc.odu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.3 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.075593 MHz
DATA PROCESSING
Line broadening 1.0 Hz
FT size 32768
Total time 1 min, 4 sec
7.318
4.731
7.5
7.0
6.5
6.0
5.5
5.0
ppm
9.59
2.89
10.13
2.97
4.00
N,N-dibenzyl-4-(10H-phenothiazin-10-yl)aniline
49

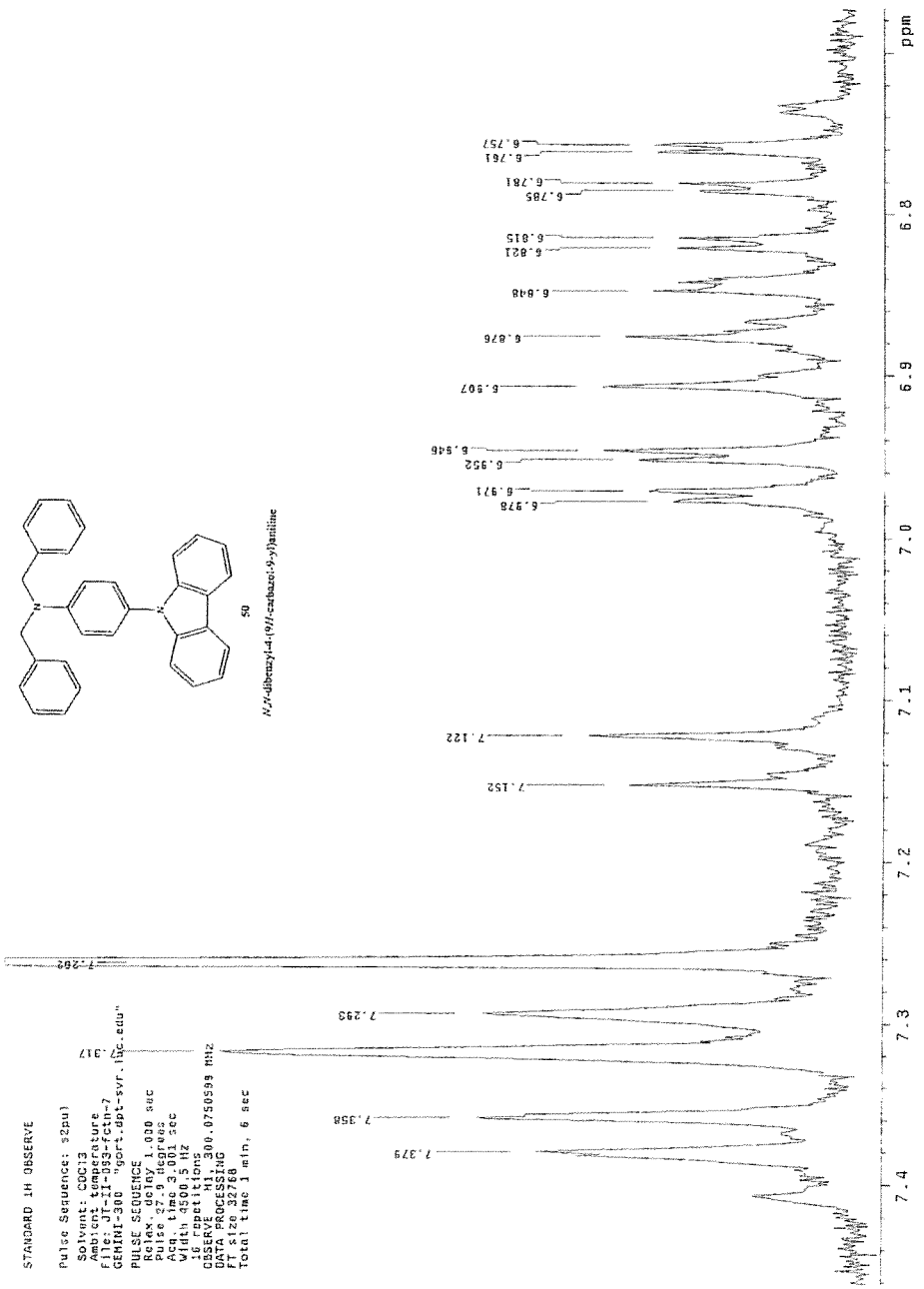

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.8 degrees
Acq. time 3.081 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750593 MHz
DATA PROCESSING
Line broadening 0.5 Hz
FT size 32768
Total time 1 min, 6 sec
49
ppm

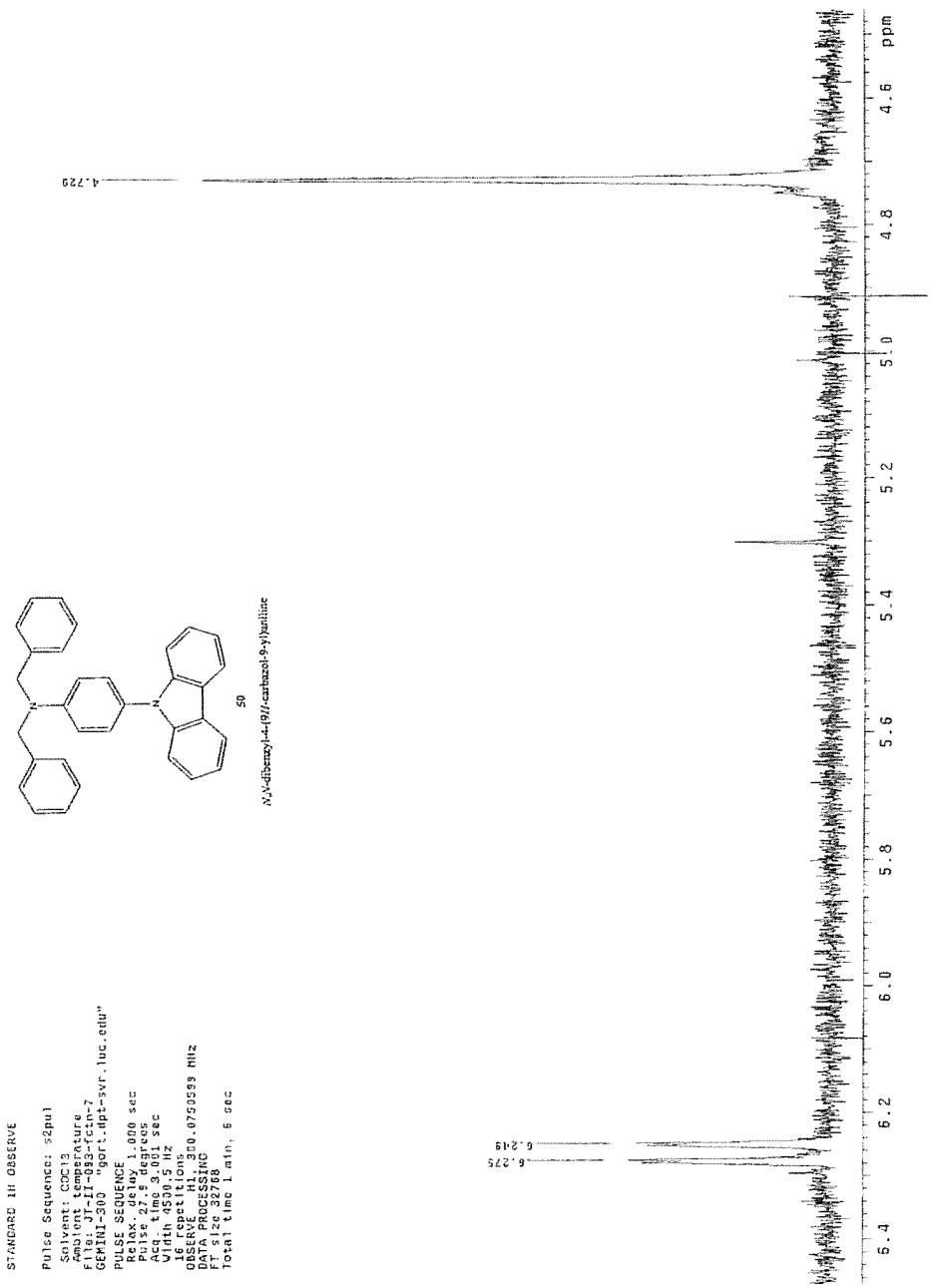

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gsrt.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 22.4 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750593 MHz
DATA PROCESSING
Line broadening 1.0 Hz
FT size 32768
Total time 1 min, 6 sec
N,N-dibenzyl-4-(10H-phenothiazin-10-yl)aniline
49
7.262
7.284
7.318
7.358
7.360
7.402
7.122
7.152
6.758
6.783
6.820
6.843
6.826
6.906
6.922
6.947
6.251
6.276
9.59
2.89
10.43
2.97
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
ppm

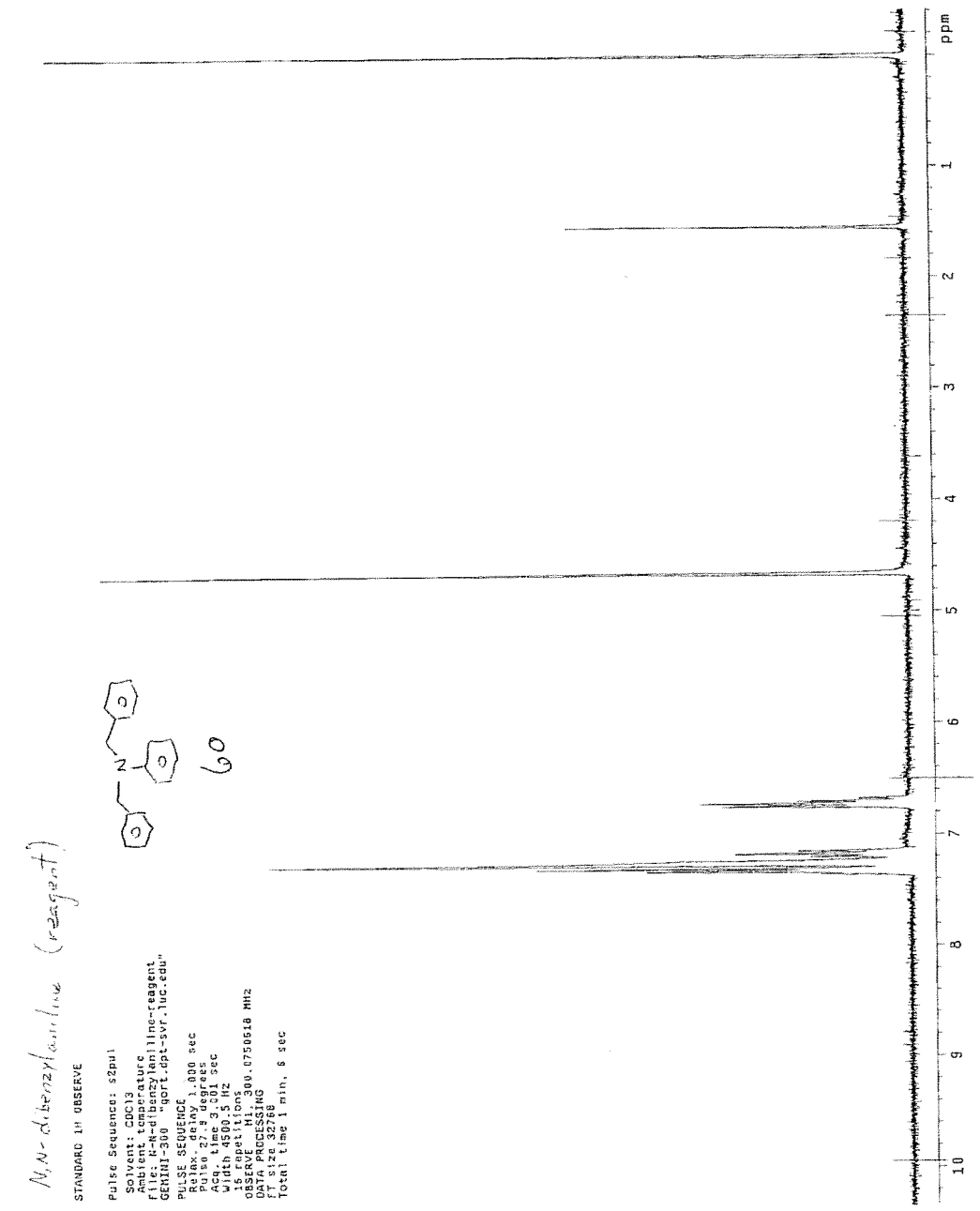

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: JT-II-093-fctn-7
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4540.5 Hz
1 repetitions
OBSERVE H1, 300.0750598 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
59
N,N-dibenzyl-4-(9H-carbazol-9-yl)aniline
ppm

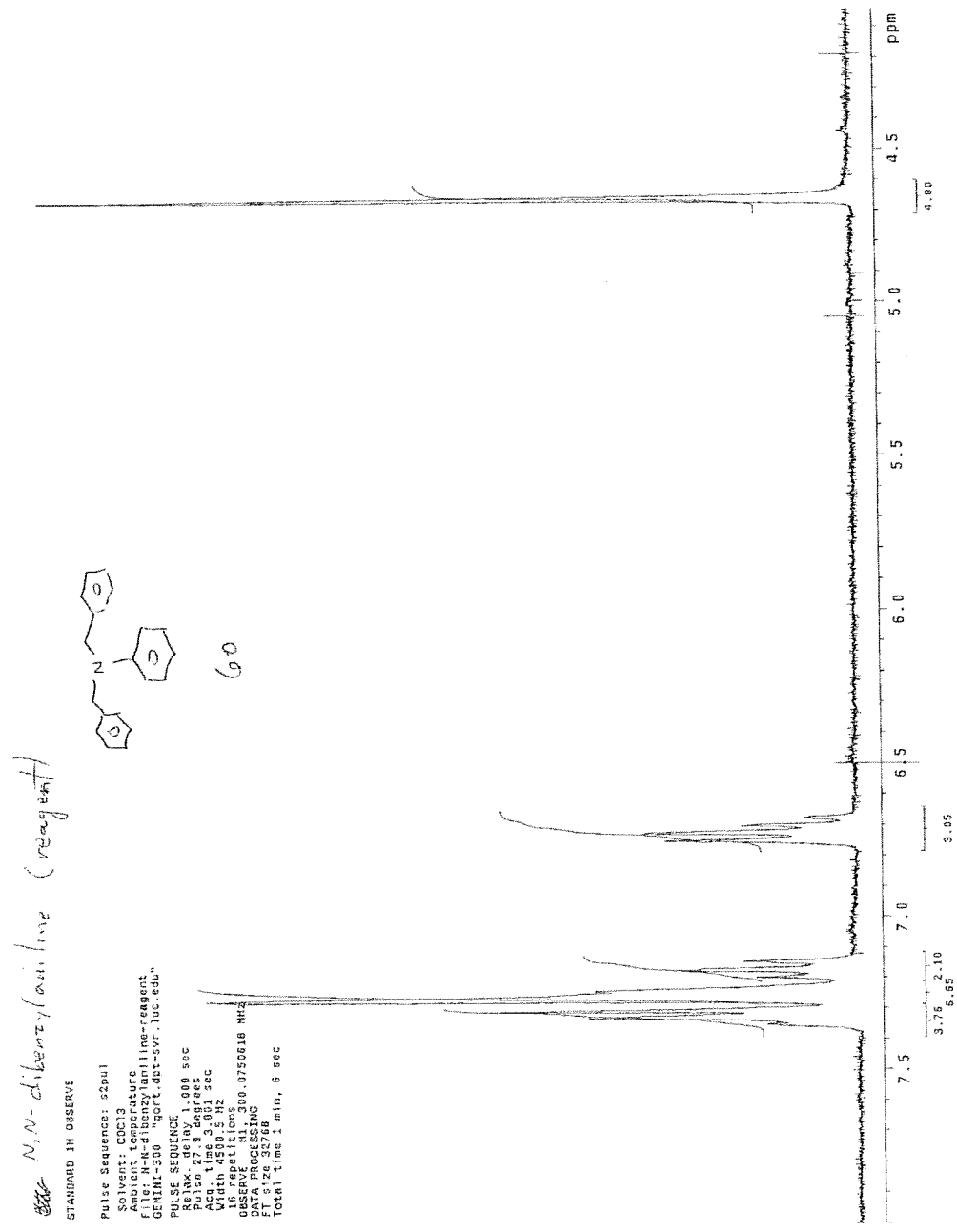

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: JT-II-093-fctn.7
GEMINI-300 "gor.dpf-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.000 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750599 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
50
N,N-dibenzyl-4-(9H)-carbazol-9-ylaniline
7.5
7.0
6.5
6.0
5.5
5.0
ppm

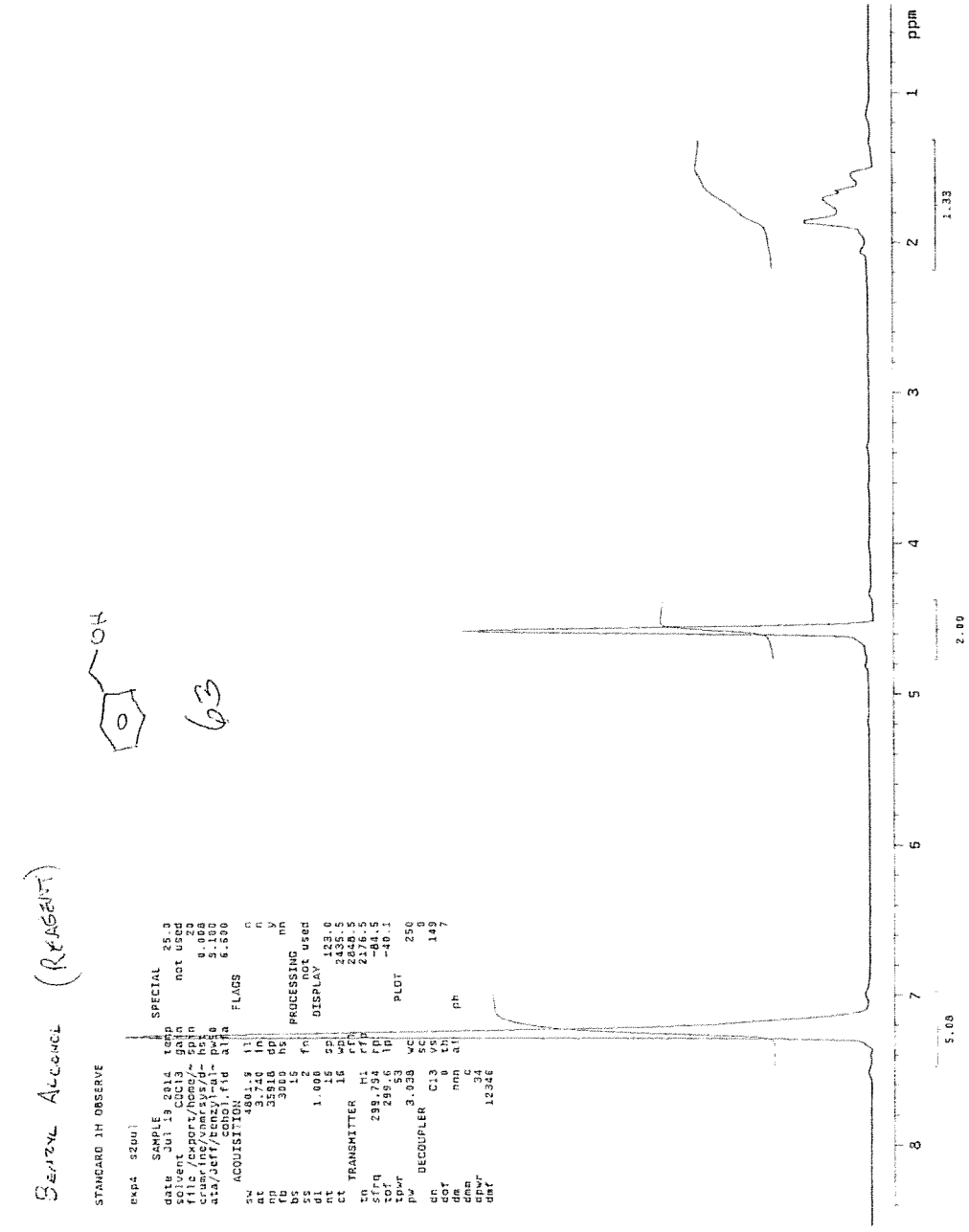

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: JT-II-093-fcth-7
GEMINI-300 "gort.dpa-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750593 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
59
N,N'-dibenzyl-4-(9H-carbazol-9-yl)aniline
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
5.4
5.2
5.0
4.8
ppm
3.67
5.32
2.12
2.61
2.55
2.15
2.31
2.20
1.00


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: JT-II-95-2th-7
GEMINI-300 "gort.dpt-svr.lsc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750599 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
7.317
7.293
7.379
7.358
7.282
7.152
7.122
6.978
6.971
6.952
6.946
6.907
6.876
6.848
6.821
6.815
6.785
6.781
6.761
6.757
7.4
7.3
7.2
7.1
7.0
6.9
6.8
ppm
50
N,N-dibenzyl-4-(9H-carbazol-9-yl)aniline


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: JT-II-083-fcln-7
GEMINI-300 "ggrt.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.8 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750599 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 5 sec
59
N,N-dibenzyl-4-(9H-carbazol-9-yl)aniline
4.729
6.279
6.249
5.4
6.0
6.2
5.8
5.6
5.4
5.2
5.0
4.8
4.6
ppm


N,N-dibenzylaniline (reagent)
STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: N-ndibenzylaniline-reagent
GEMINI-300 "qcrf.cpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 22.4 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750618 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
N
60
ppm
10
9
8
7
6
5
4
3
2
1


N,N-dibenzylaniline (reagent)
60
STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: N-N-dibenzylaniline-reagent
GEMINI-300 "port.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750018 MHz
DATA PROCESSING
FT 32768
Total time 1 min, 6 sec
7.5
7.0
6.5
6.0
5.5
5.0
4.5
ppm
3.75
2.10
5.85
3.05
4.00


Benzyl Alcohol (REAGENT)
OH
O
G3
STANDARD 1H OBSERVE
expt s2pul
SAMPLE
date Jul 19 2014
solvent CDCl3
file /export/home/~
crunching/vnmrsys/g-
ata/jeff/benzyl-al-
CDCl3.fid
ACQUISITION
sw 4801.9
at 3.740
np 35918
fb 3000
bs 16
ss 2
nt 1.000
ct 16
TRANSMITTER
tn H1
sfrq 299.734
tof 299.6
tpwr 53
pw 3.038
DECOUPLER
dn C13
dof 0
dm nnn
dmm c
dmf 34
dmf 12346
SPECIAL
temp 25.0
gain not used
spin 20
hst 0.008
pw90 5.180
alfa 6.630
FLAGS
il n
in n
dp y
hs nn
PROCESSING
not used
DISPLAY
sp 123.0
wp 2435.5
rfl 2848.5
rfp 2176.5
rp -84.5
lp -40.1
PLOT
wc 250
sc 0
vs 149
th 7
ph
af
5.08
2.00
1.33
ppm

Benzyl Alcohol (Reagent)

63

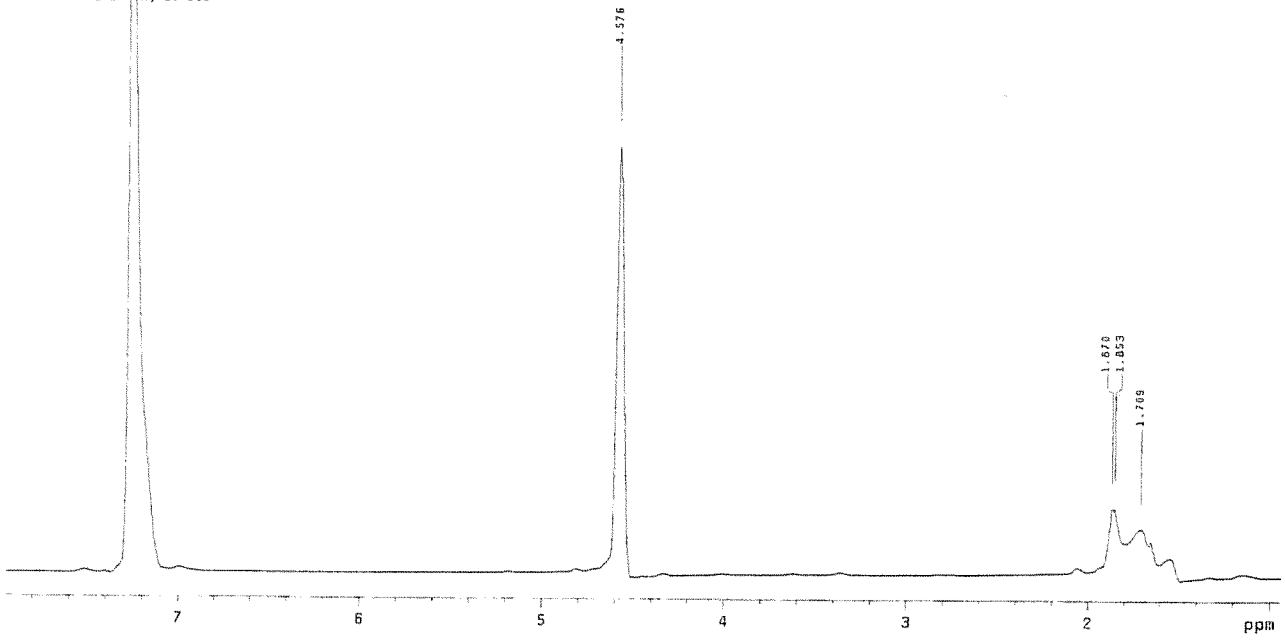
STANDARD 1H OBSERVE

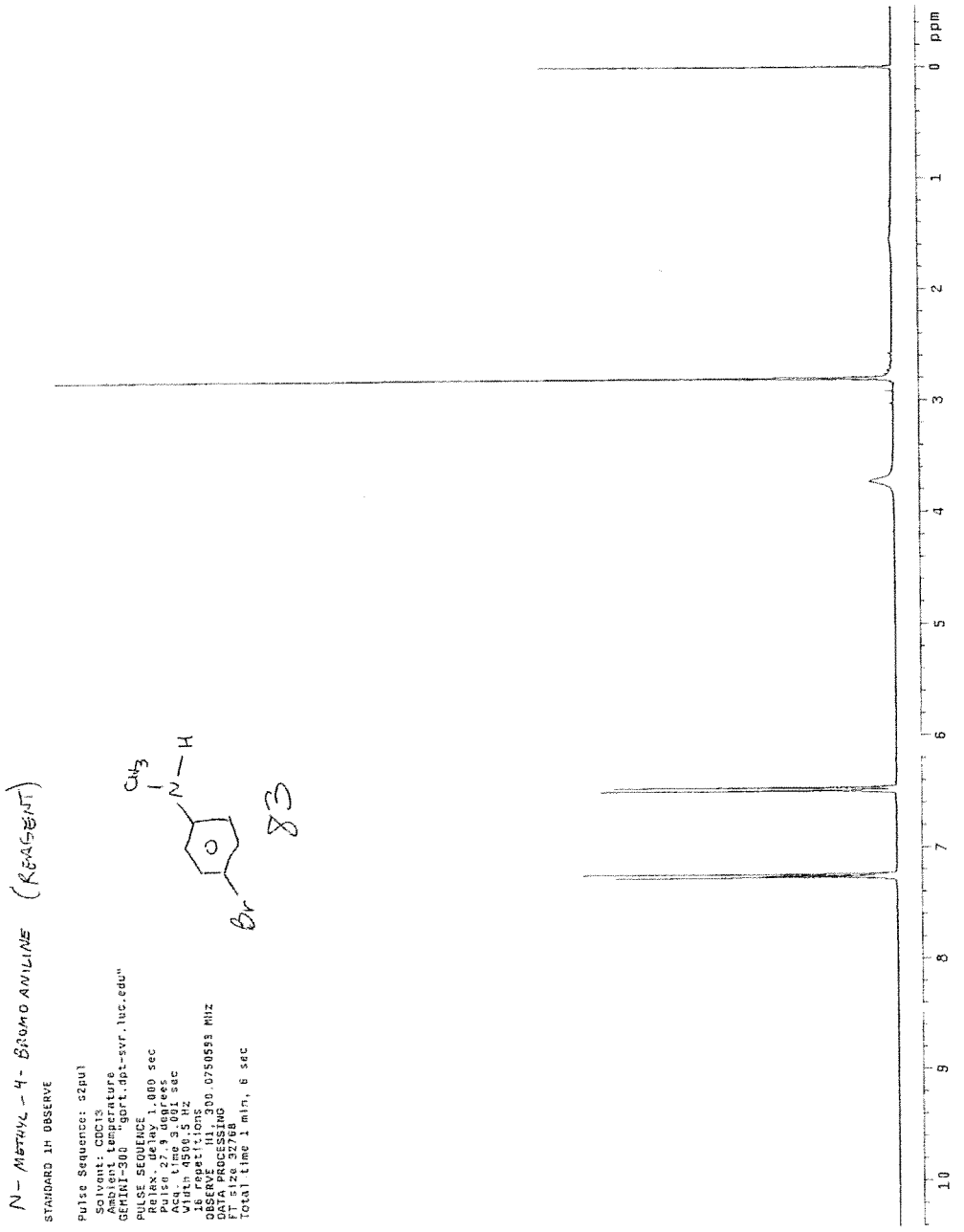
Archive directory: /export/home/vnmr1/vnmrsys/data
Sample directory:

Pulse Sequence: s2pul

Solvent: CDCl3
Temp. 25.0 C / 298.1 K
File: benzyl-alcohol
INOVA-300 "rodan.dpt-svr.luc.edu"

Relax. delay 1.000 sec
Pulse 30.0 degrees
Acq. time 3.220 sec
Width 4861.1 Hz
16 repetitions
OBSERVE H1, 299.7527580 MHz
DATA PROCESSING
FT size 65536
Total time 1 min, 25 sec

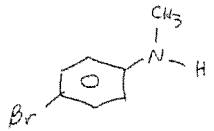



N- METHYL - 4- BROMO ANILINE (REAGENT)
STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.091 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750593 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
CH3
N
H
Br
83
10
9
8
7
6
5
4
3
2
1
0 ppm

N-METHYL-4-BROMOANILINE (REAGENT)

STANDARD IH OBSERVE

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4590.5 Hz
16 repetitions
OBSERVE H1, 300.0750599 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec

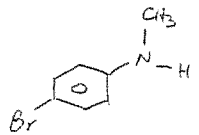


83

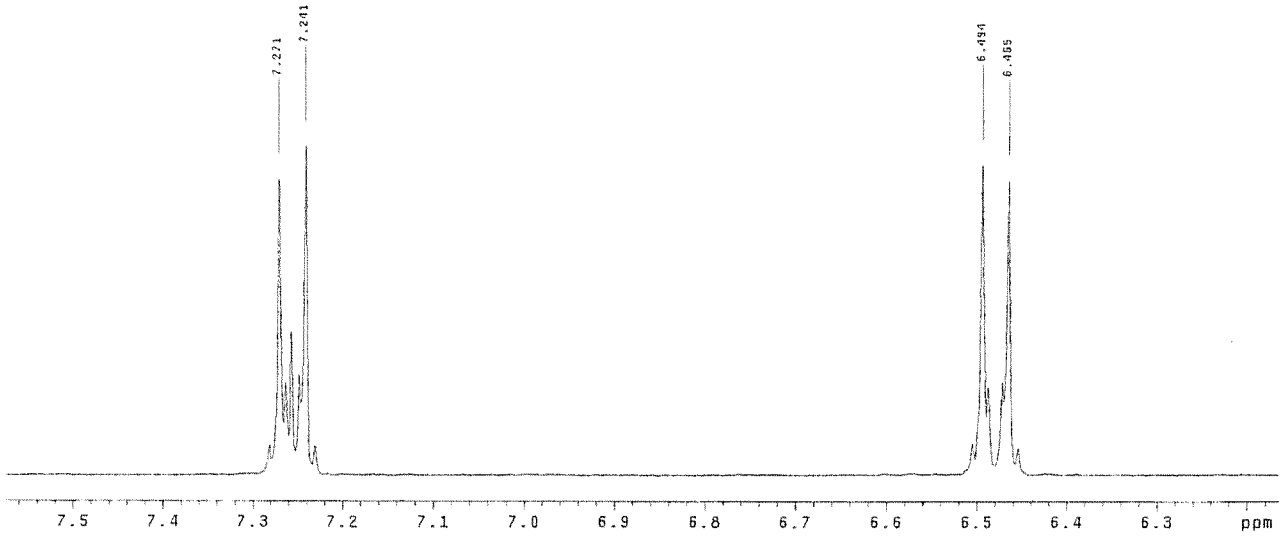
N-METHYL-4-BROMOANILINE (REAGENT)

STANDARD 1H OBSERVE

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.iuc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750599 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec



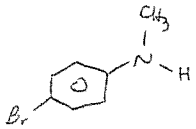
83



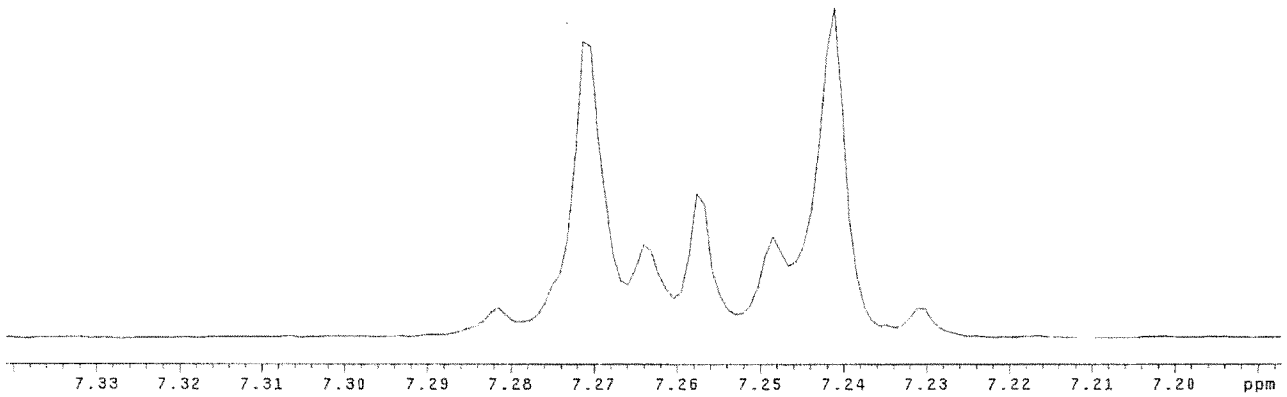
N-METHYL-4-BROMOANILINE (REAGENT)

STANDARD 1H OBSERVE

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750599 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec



83

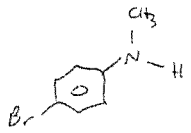


N-METHYL-4-BROMO ANILINE (REAGENT)

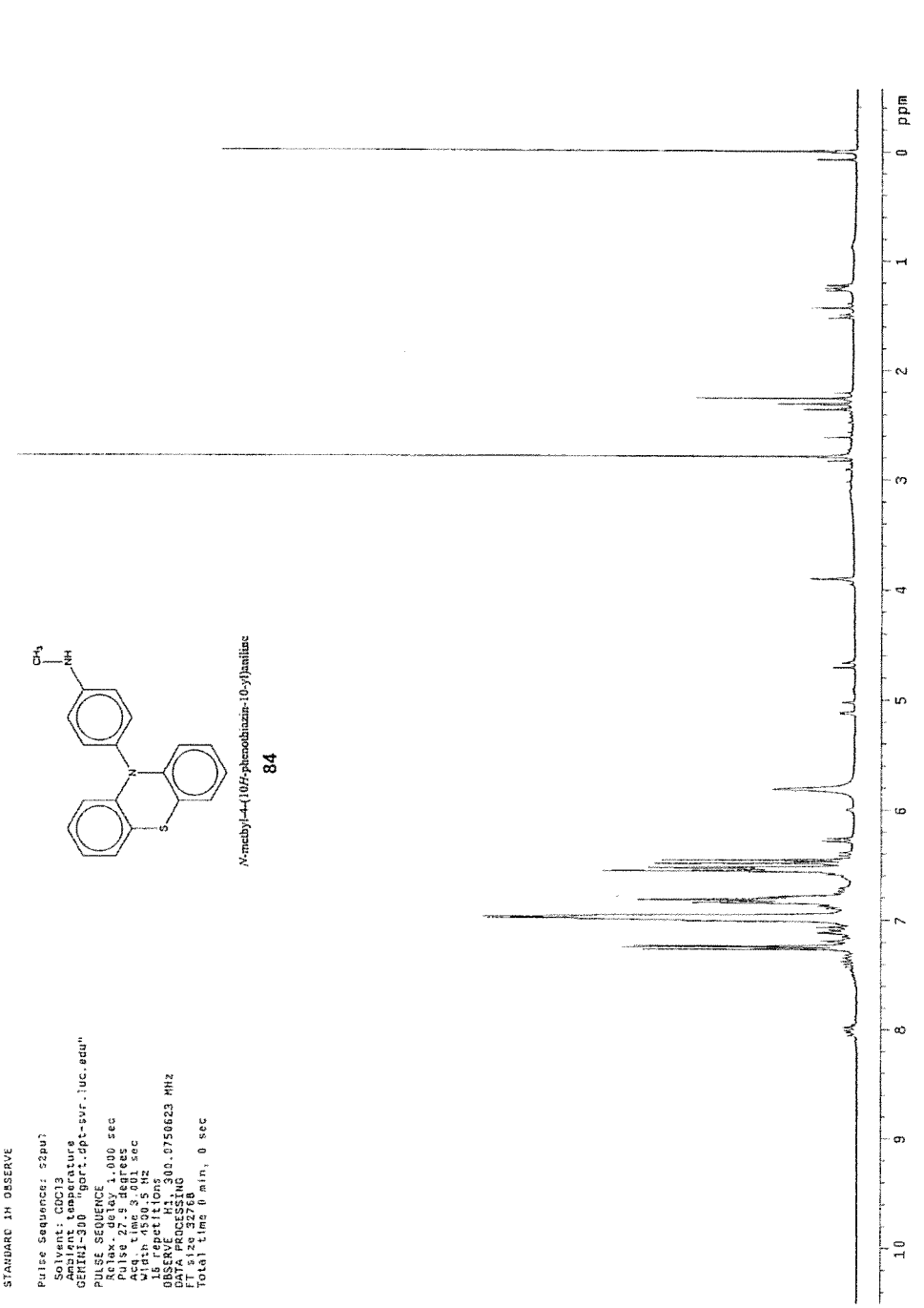
STANDARD 1H OBSERVE

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"

PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.5 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
18 repetitions
OBSERVE H1, 300.0750599 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec

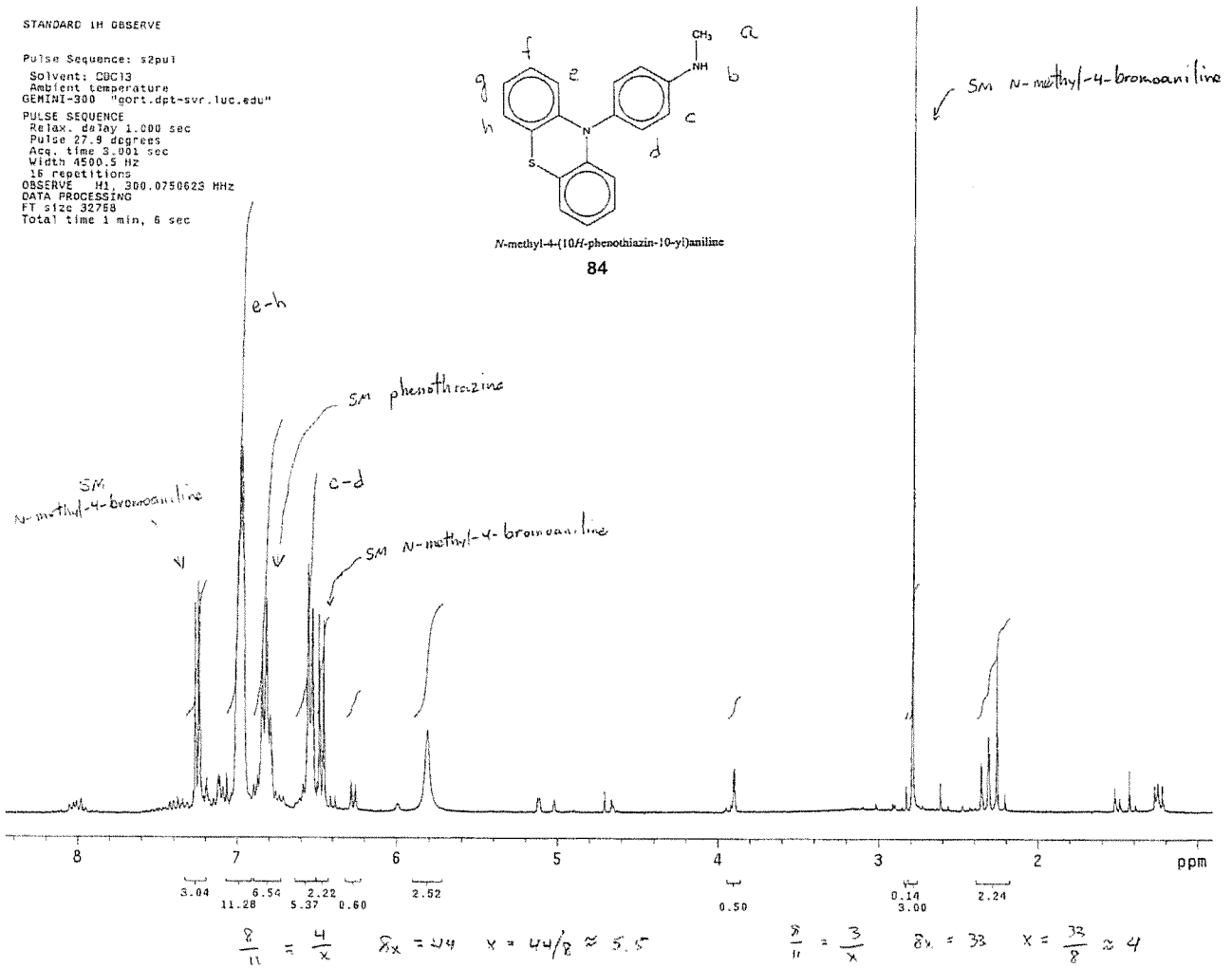


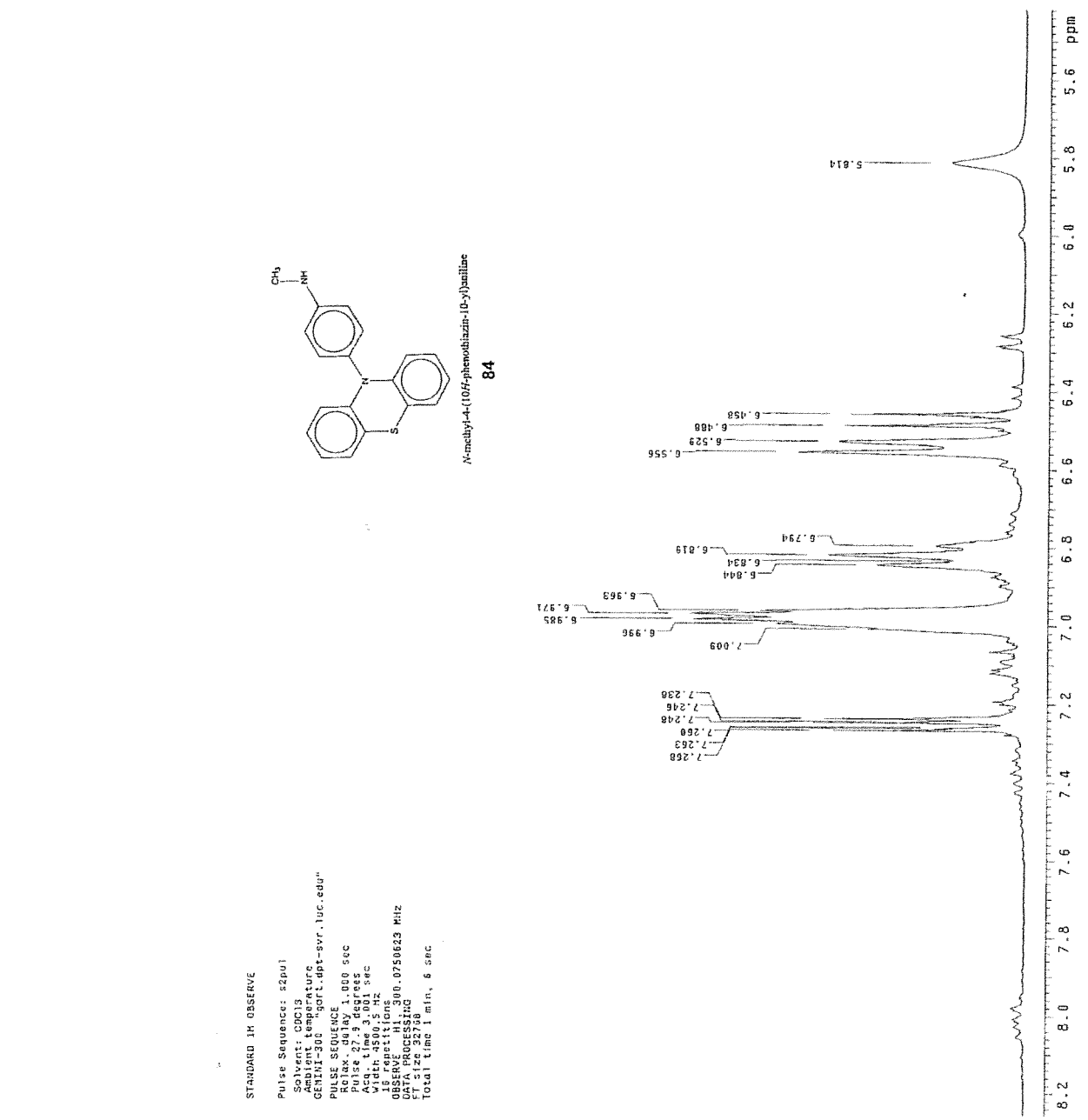
83

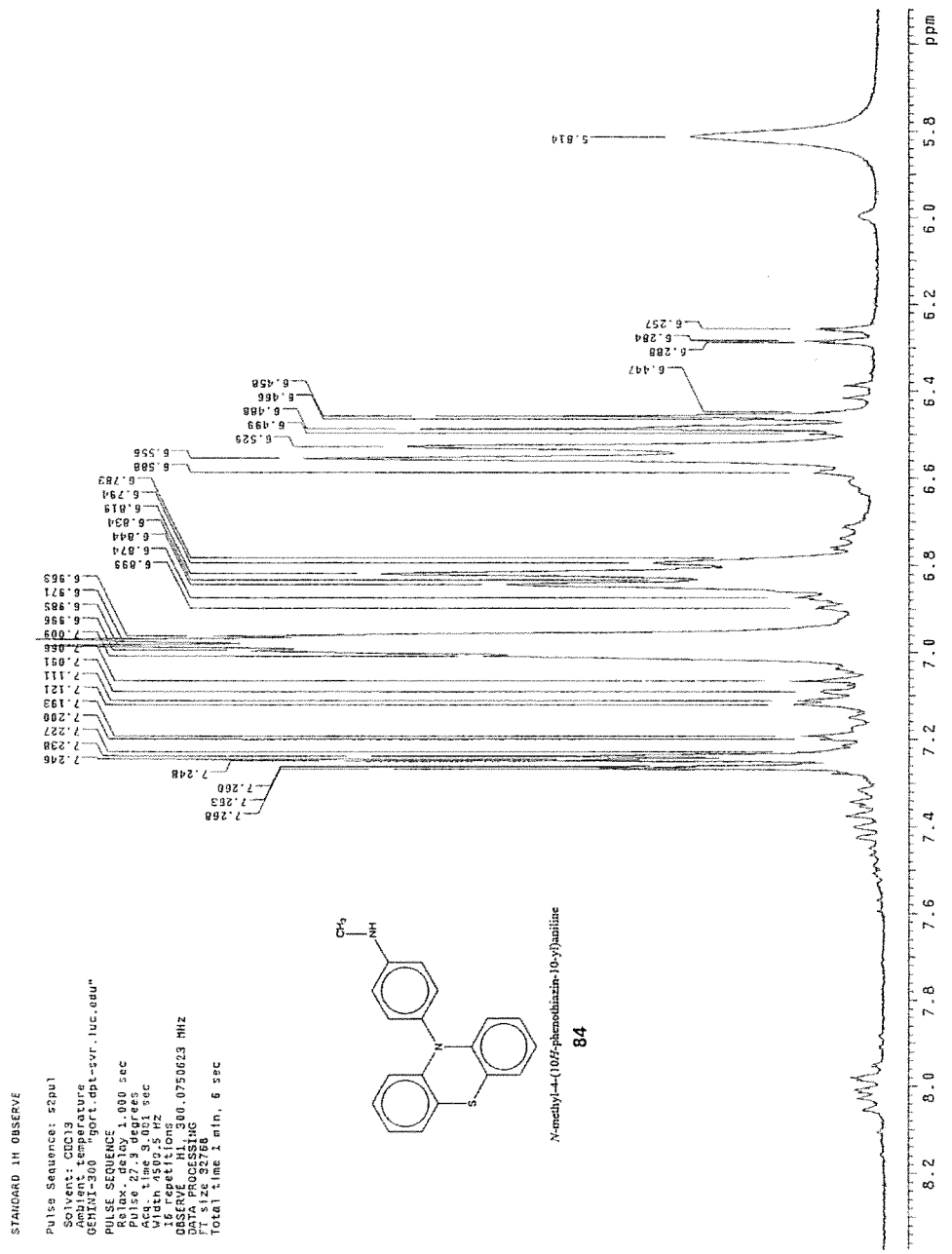


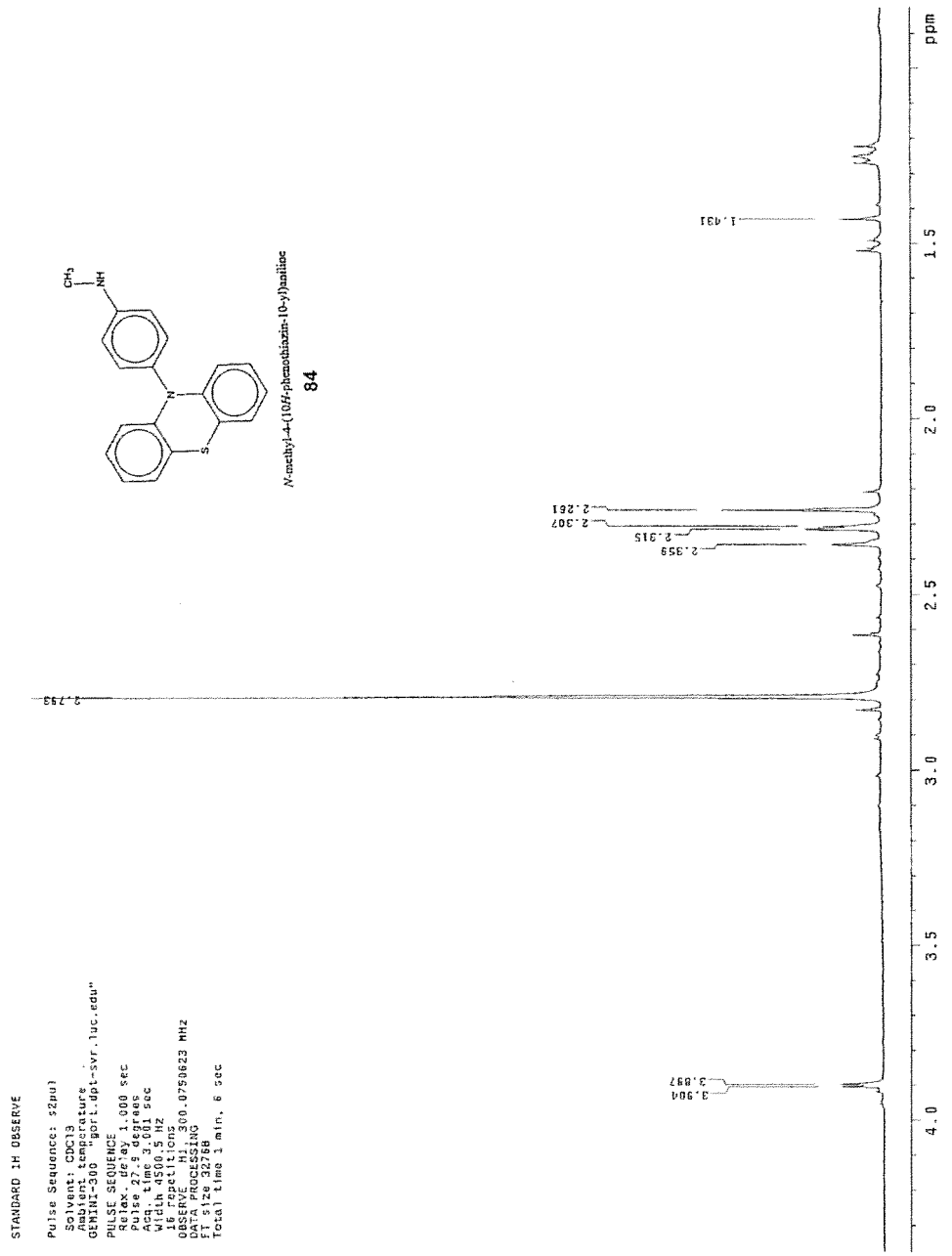
N-methyl-4-(10*H*-phenothiazin-10-yl)aniline

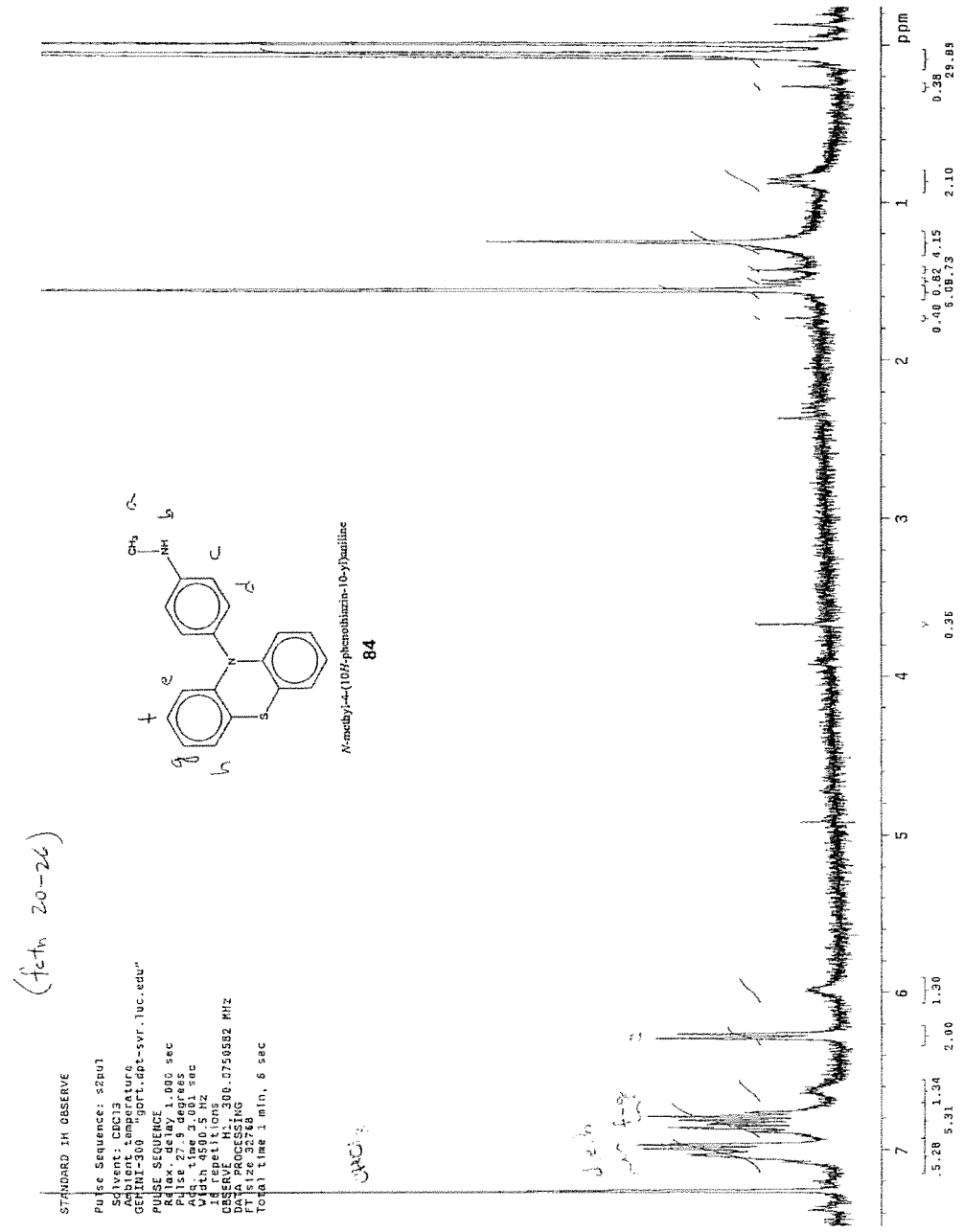
84


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750623 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
N-methyl-4-(10H-phenothiazin-10-yl)aniline
84
SM N-methyl-4-bromoaniline
e-h
SM phenothiazine
c-d
SM N-methyl-4-bromoaniline
SM N-methyl-4-bromoaniline
8
7
6
5
4
3
2
ppm
3.04
6.54
11.28
2.22
5.37
0.60
2.52
0.50
0.14
3.00
2.24
8/11 = 4/x δx = 24 x = 44/8 ≈ 5.5
3/11 = 3/x δx = 33 x = 33/8 ≈ 4


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.lsu.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4810.5 Hz
16 repetitions
OBSERVE H1, 300.0750623 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
CH3
NH
N
S
84
N-methyl-4-(10H-phenothiazin-10-yl)aniline
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
5.6
ppm
7.268
7.263
7.260
7.248
7.246
7.236
7.009
6.996
6.985
6.971
6.963
6.844
6.834
6.819
6.794
6.556
6.529
6.488
6.458
5.814

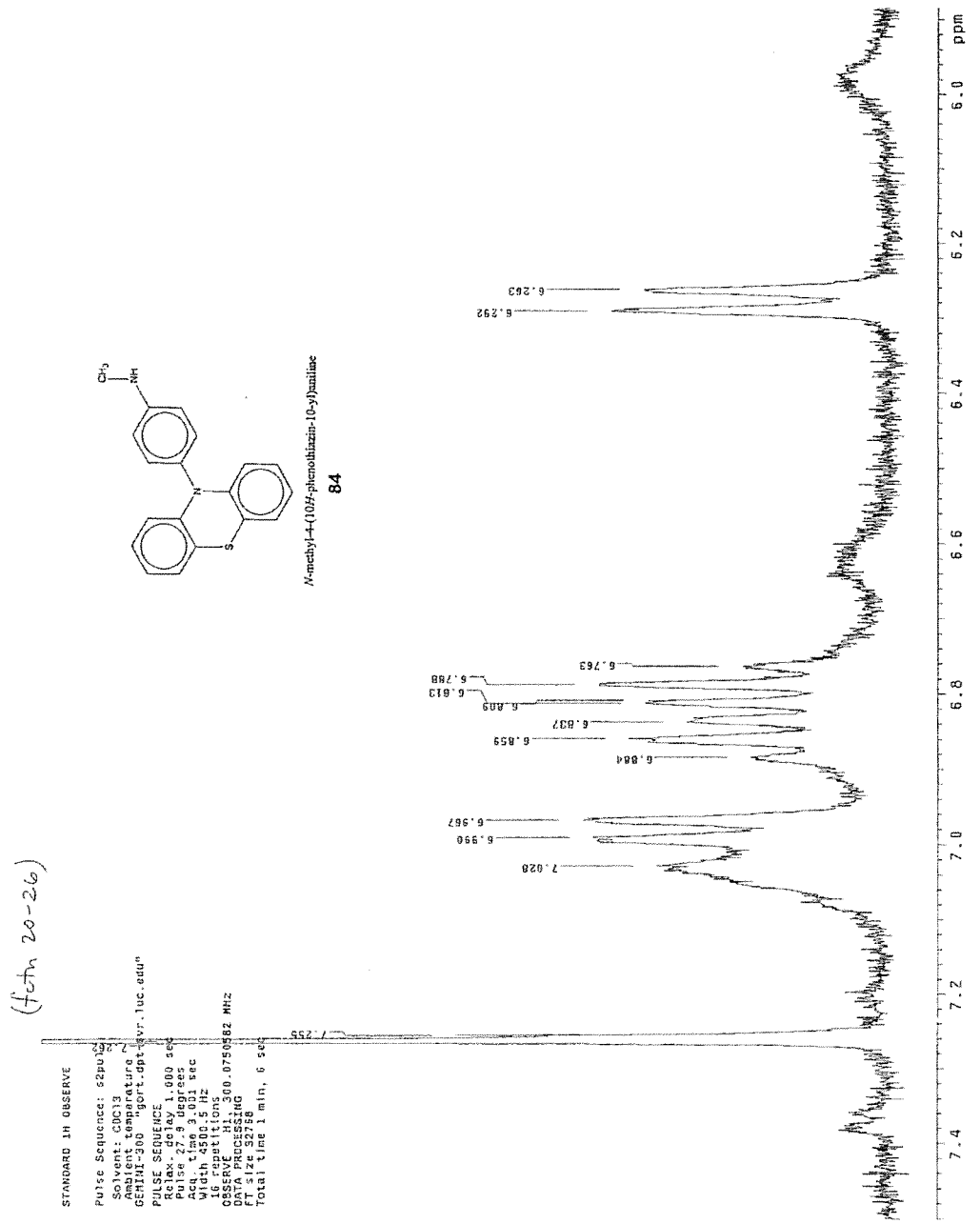

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.051 sec
Width 4509.5 Hz
1 repetitions
OBSERVE H1, 300.0750623 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
7.268
7.263
7.260
7.248
7.246
7.238
7.227
7.200
7.193
7.121
7.111
7.051
7.009
6.996
6.985
6.971
6.965
6.899
6.874
6.844
6.834
6.819
6.794
6.783
6.588
6.556
6.525
6.499
6.488
6.466
6.458
6.442
6.288
6.284
6.257
5.814
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
5.8
ppm
N-methyl-4-(10H-phenothiazin-10-yl)aniline
84

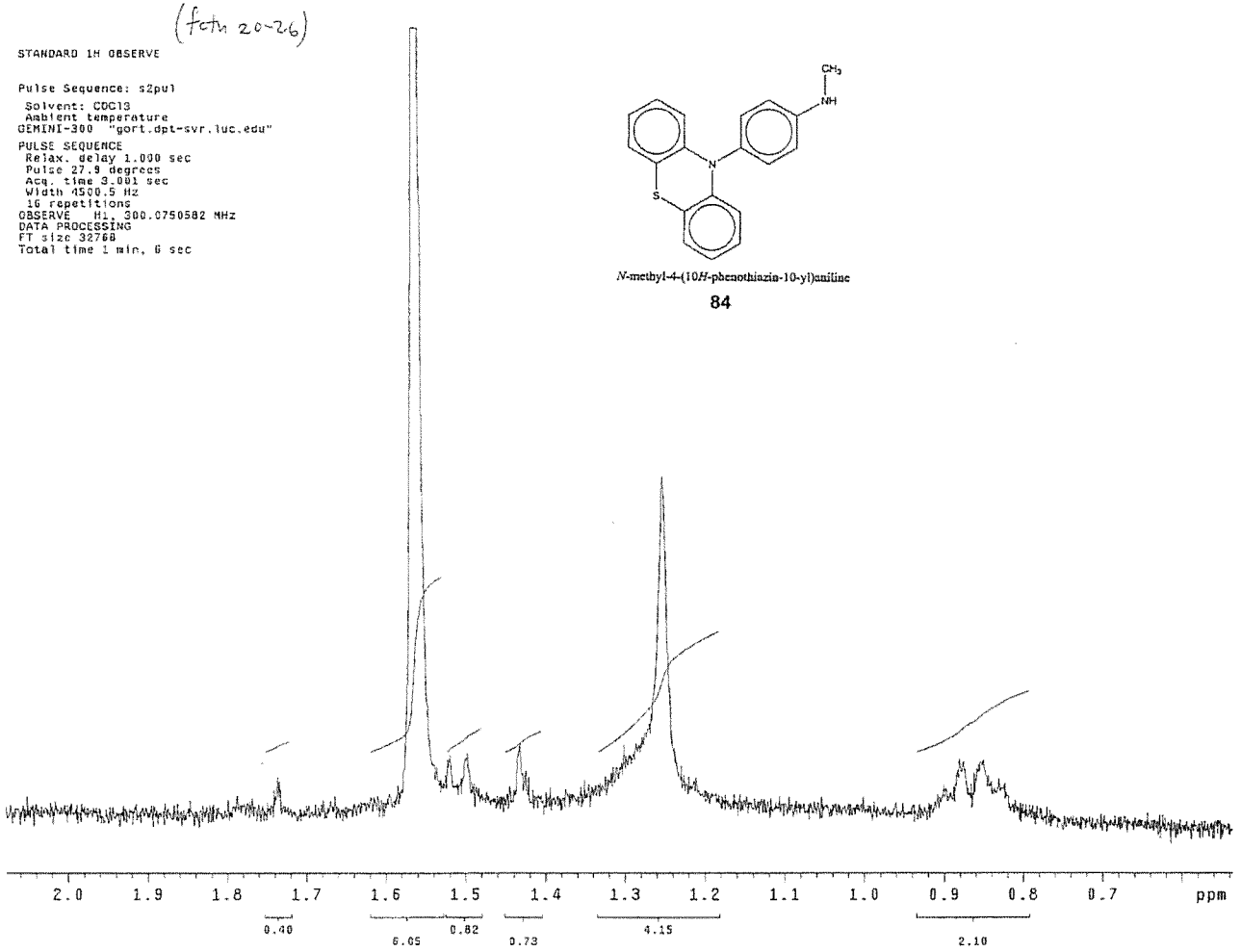

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gori.dpi-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.8 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750623 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
CH3
NH
N
S
N-methyl-4-(10H-phenothiazin-10-yl)aniline
84
2.793
3.904
3.897
2.359
2.315
2.307
2.261
1.931
4.0
3.5
3.0
2.5
2.0
1.5
ppm

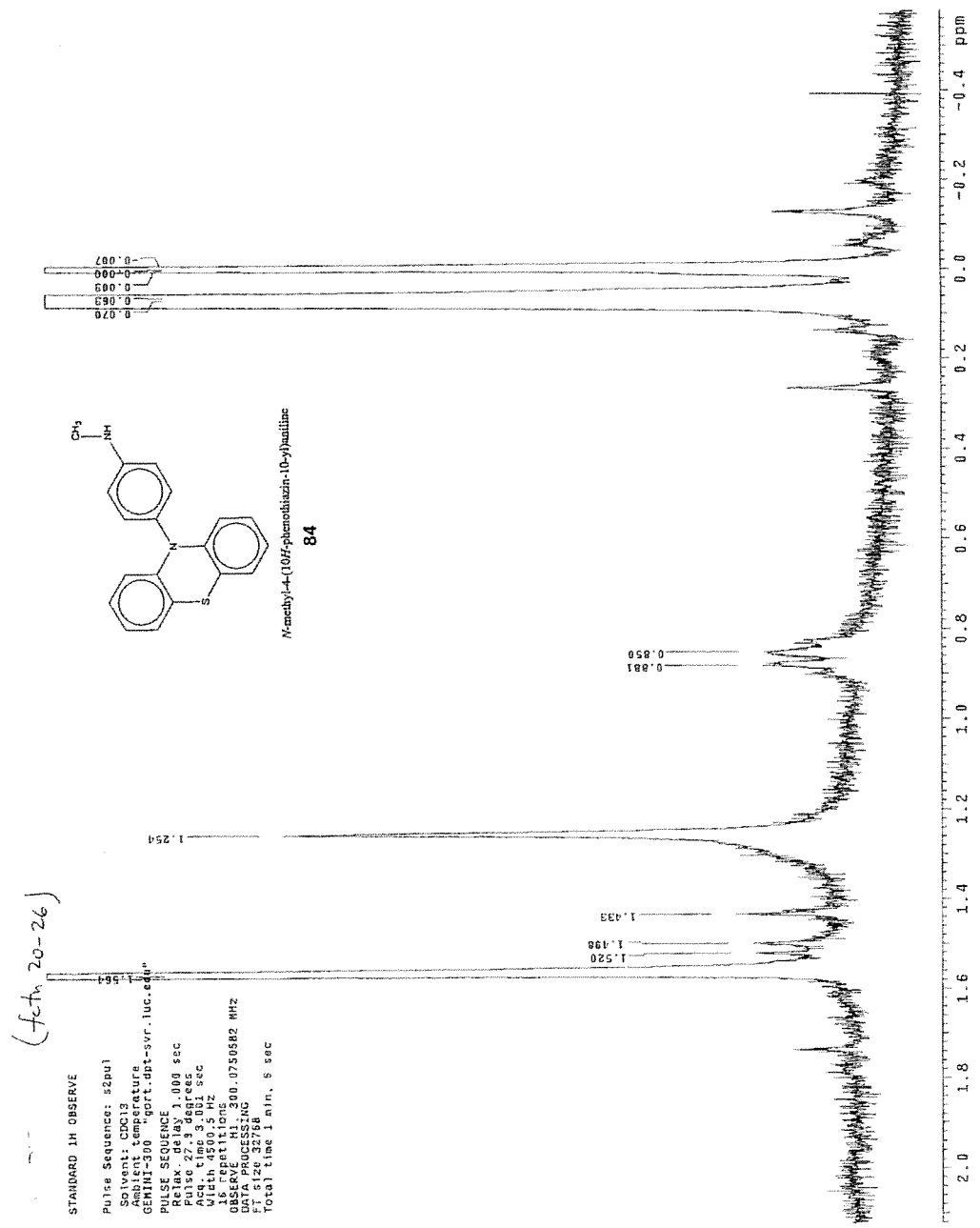


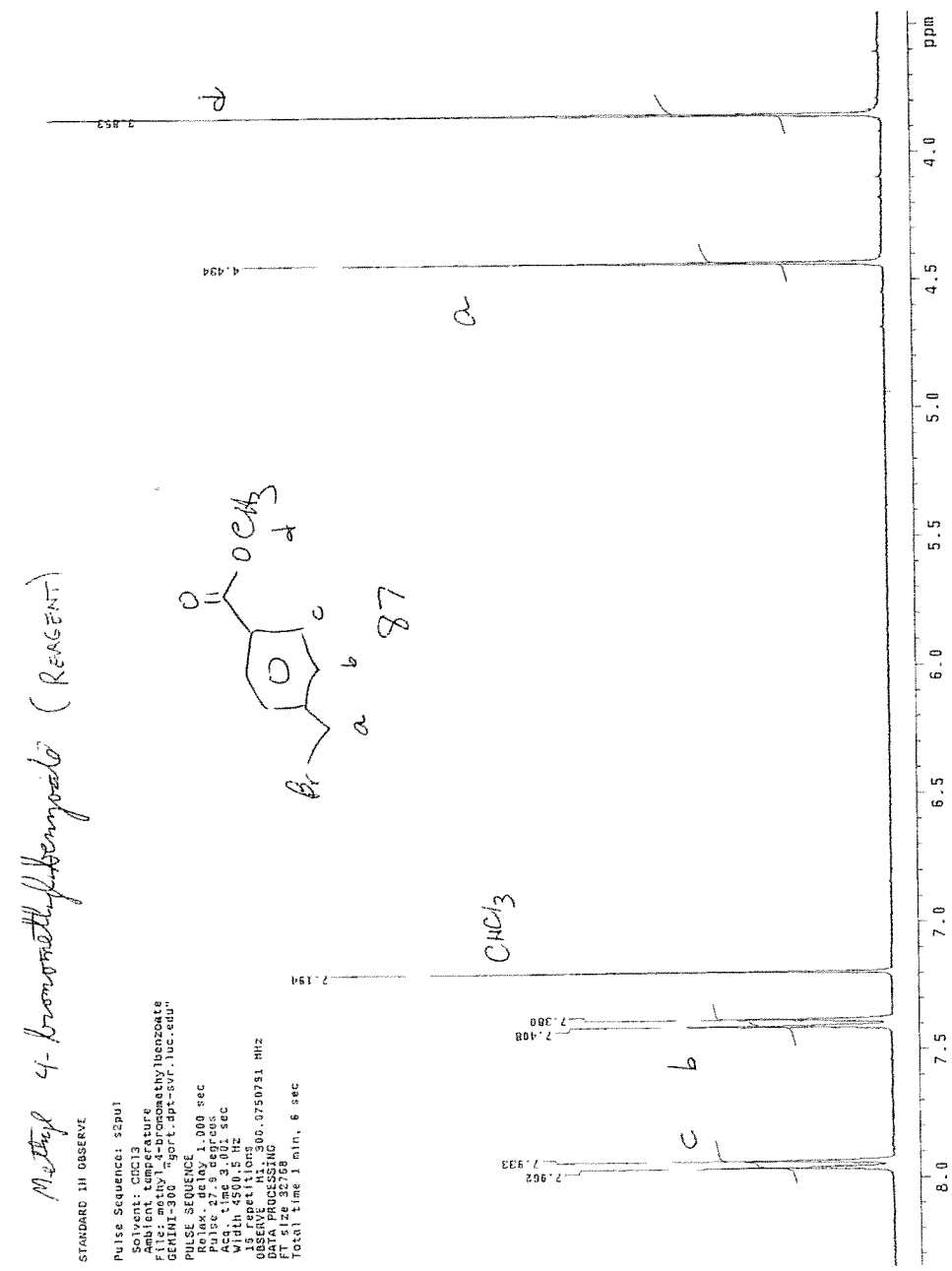
N-methyl-4-(10*H*-phenothiazin-10-yl)aniline

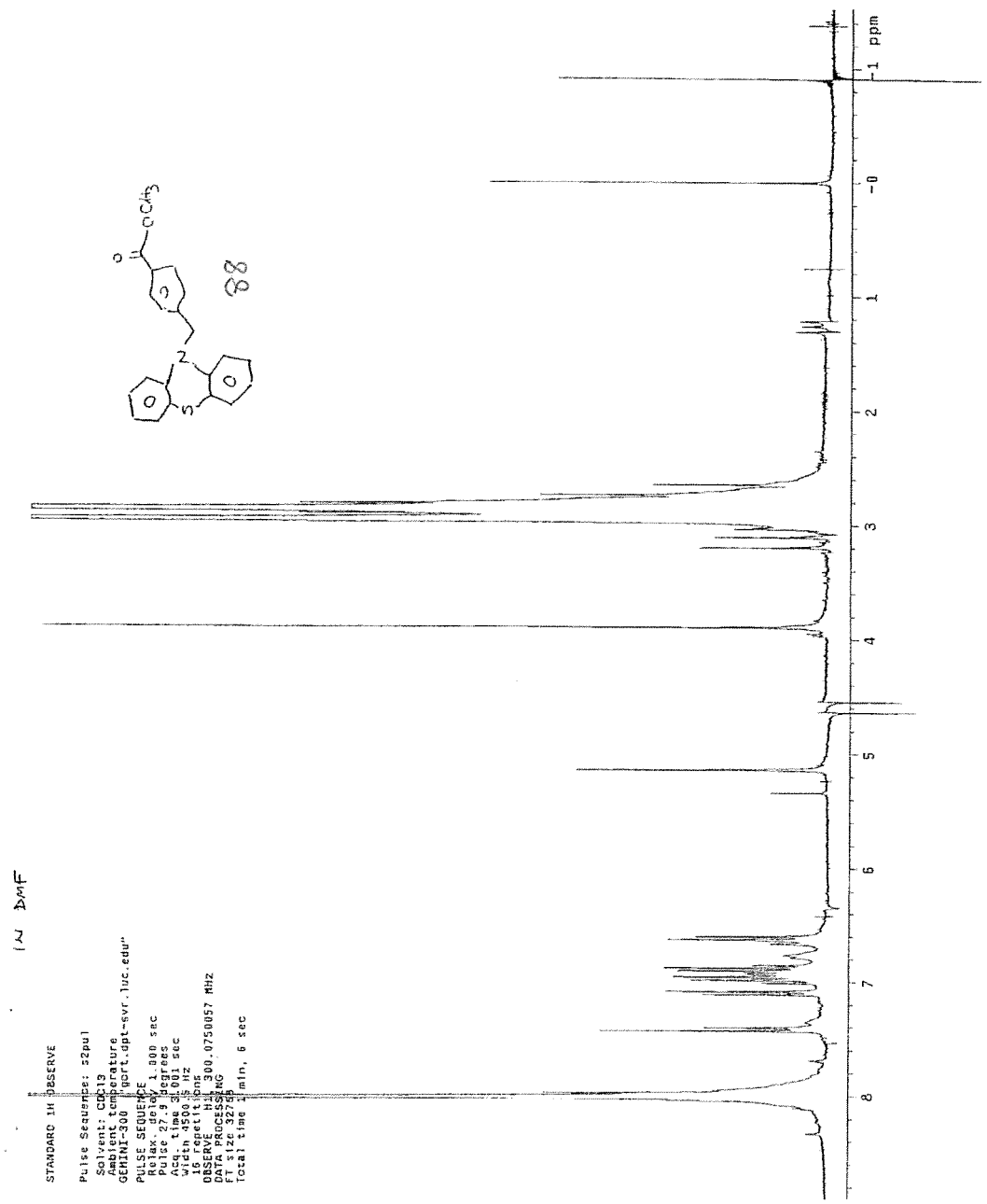
84

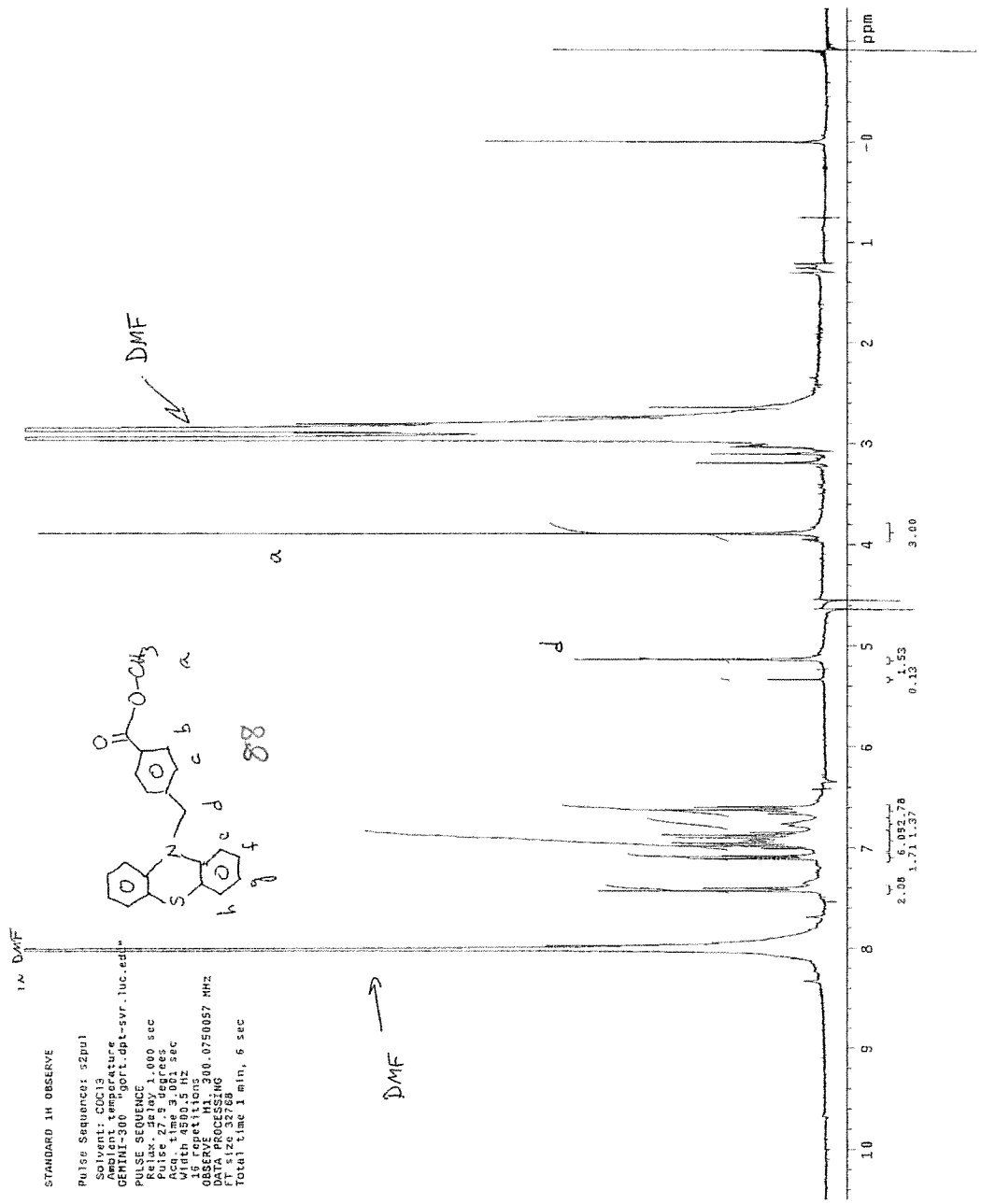

(fth 20-26)
STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750582 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
7.255
7.028
6.990
6.967
6.864
6.859
6.837
6.809
6.813
6.788
6.763
6.292
6.263
7.4
7.2
7.0
6.8
6.6
6.4
6.2
6.0
ppm
N-methyl-4-(10H-phenothiazin-10-yl)aniline
84


(fctn 20-26)
STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750582 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
CH3
NH
N
S
N-methyl-4-(10H-phenothiazin-10-yl)aniline
84
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
ppm
0.40
6.05
0.82
0.73
4.15
2.10


(fcth 20-26)
STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.801 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750582 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
1.564
1.254
1.433
1.198
1.520
0.881
0.850
0.070
0.063
0.089
0.080
0.087
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
ppm
N-methyl-4-(10H-phenothiazin-10-yl)aniline
84


Methyl 4-bromomethylbenzoate (REAGENT)
STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: methyl 4-bromomethylbenzoate
GEMINI-300 "mgort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750791 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
7.853
4.494
7.194
7.962
7.933
7.408
7.380
CHCl3
a
b
c
d
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
ppm


1N DMF
STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.901 sec
Width 4500.0 Hz
16 repetitions
OBSERVE H1, 300.0750057 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
88
O
OCH3
O
S
O
2
8
7
6
5
4
3
2
1
-0
1 ppm

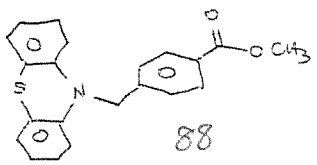

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
1 repetitions
OBSERVE H1, 300.0750057 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
1.00 DMF
DMF
DMF
a
d
ppm
2.08
6.092 7.78
1.71 1.37
1.53
0.13
3.00

IN DMF

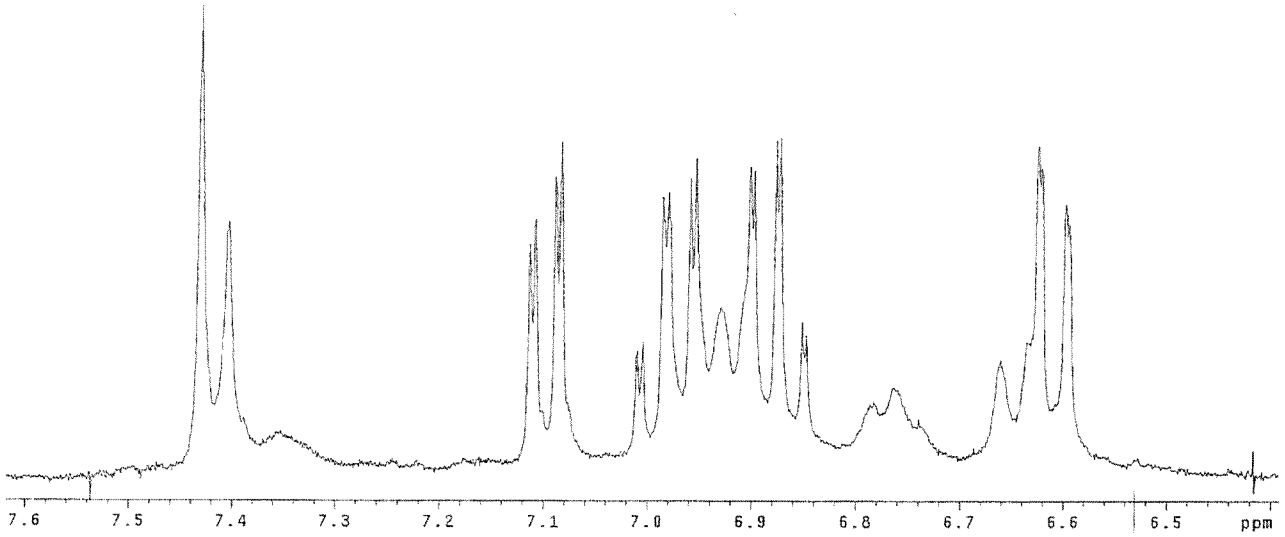
STANDARD 1H OBSERVE

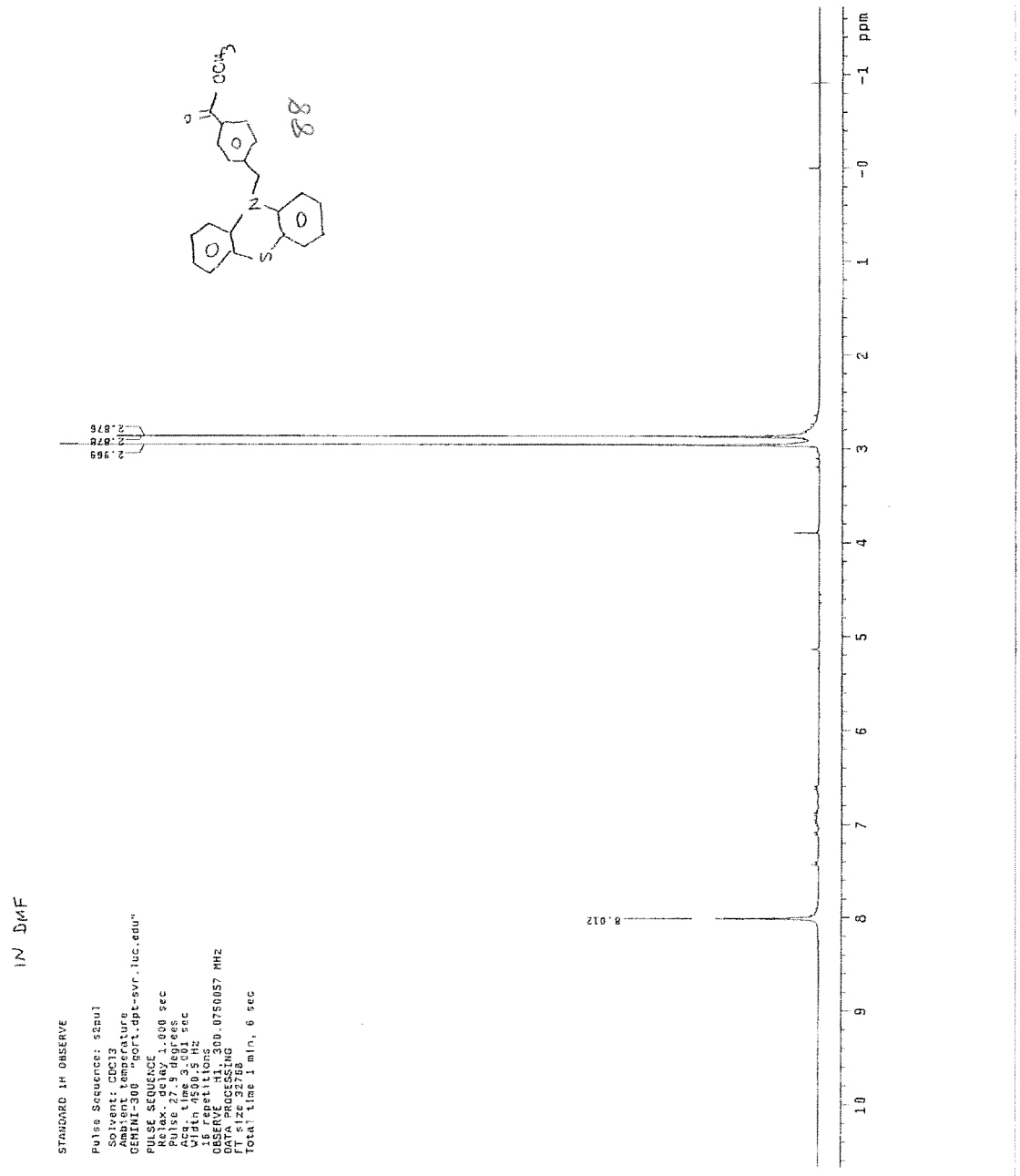
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"

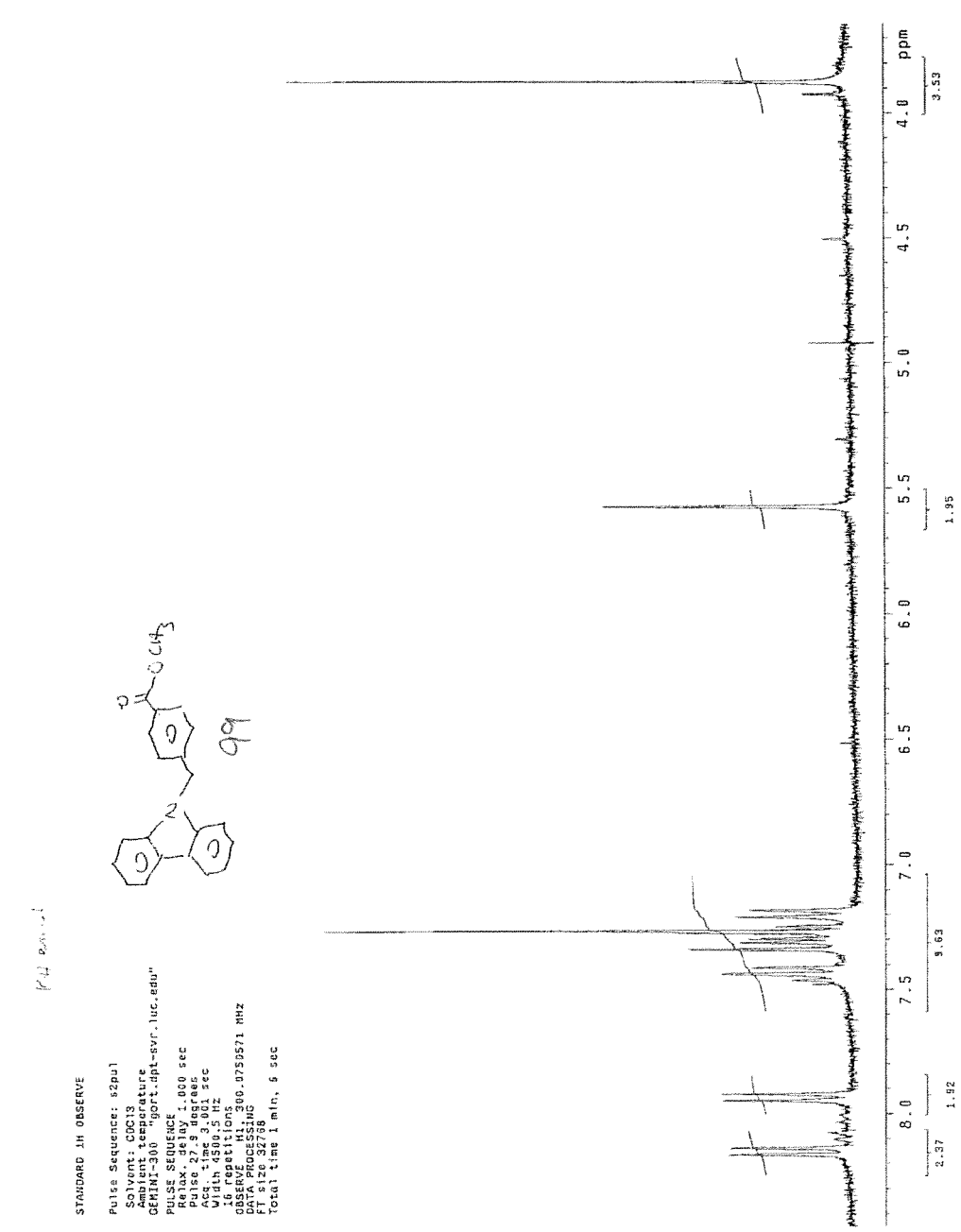
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.5 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750057 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec

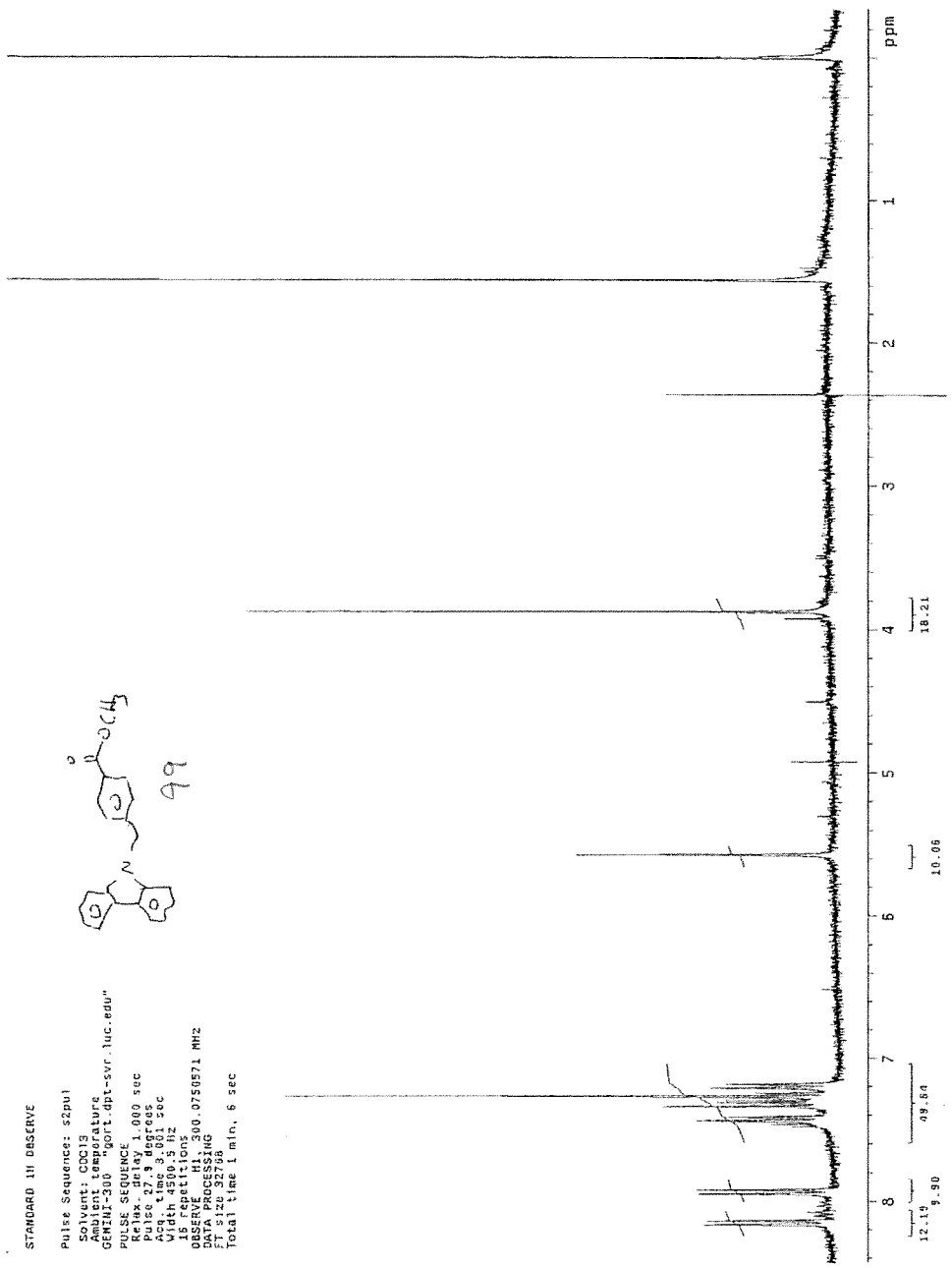


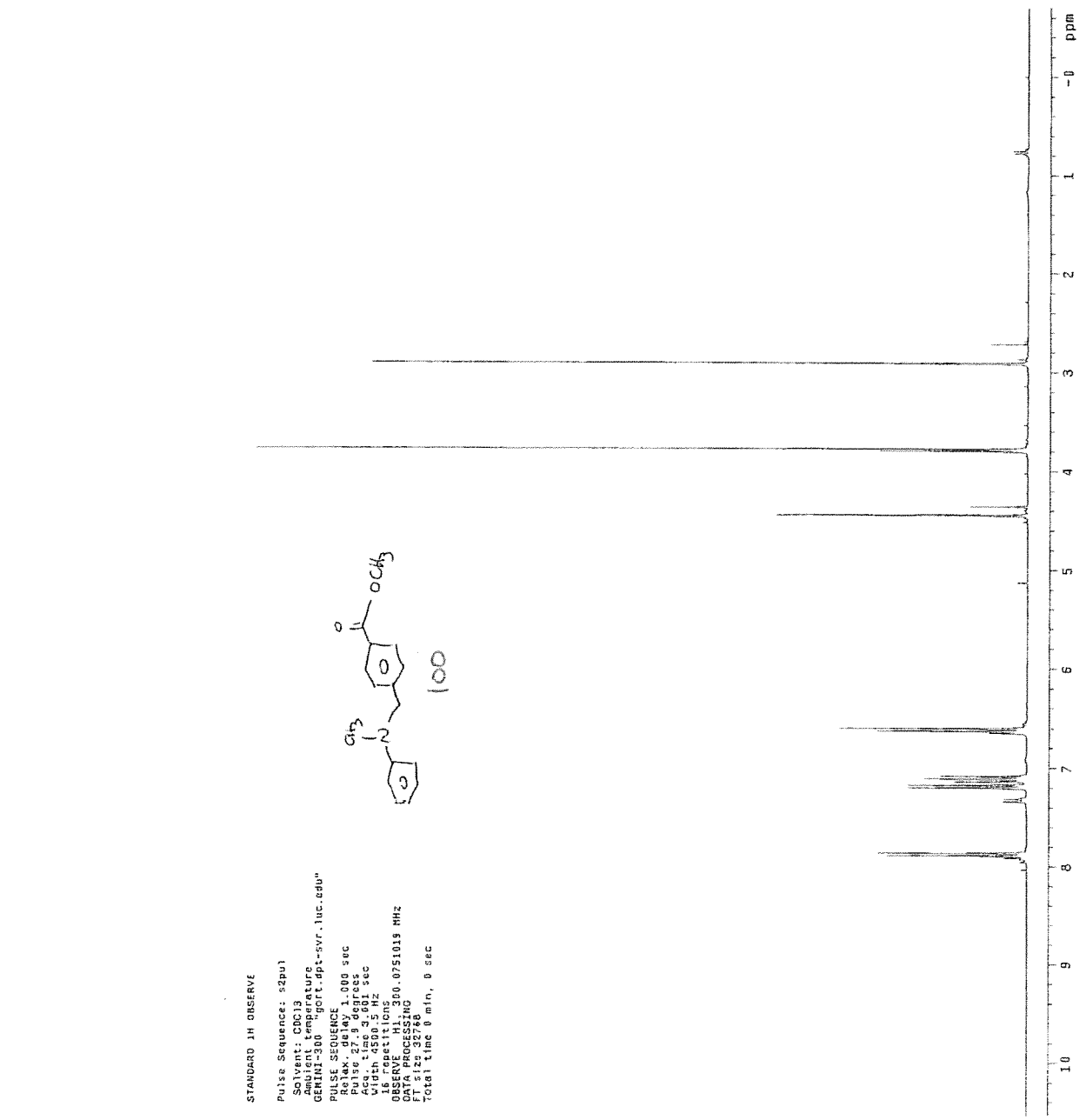
88

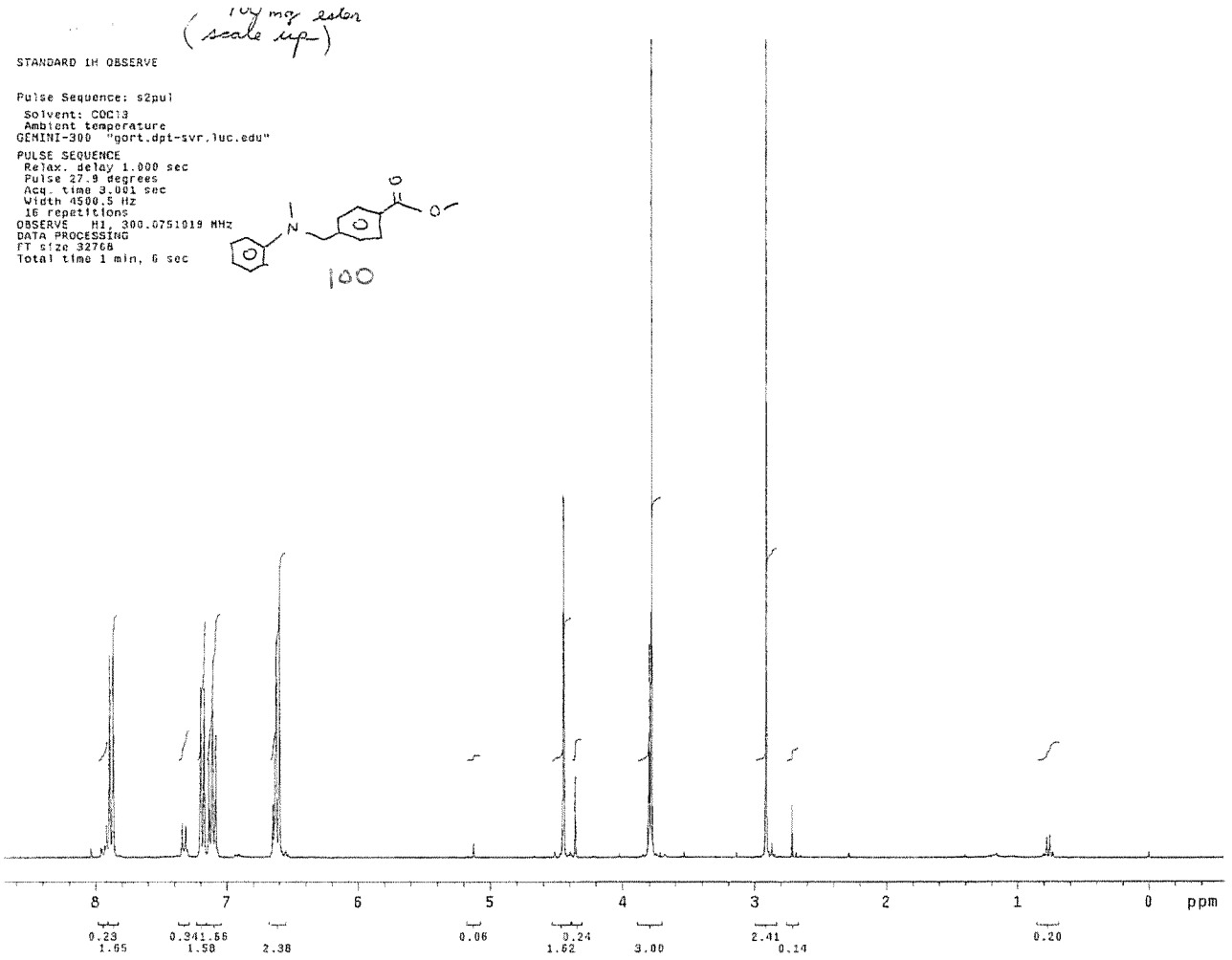


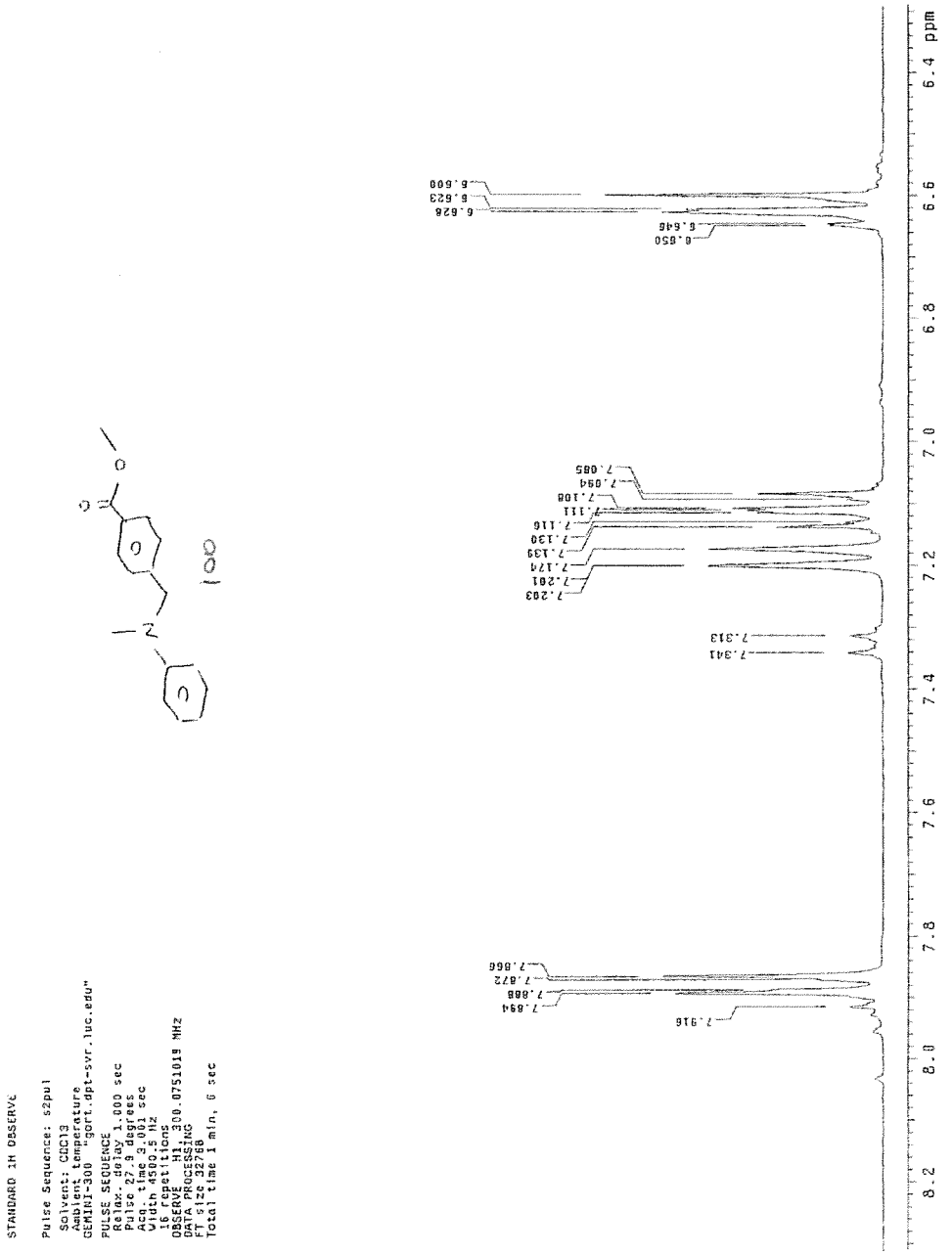

IN DMF
STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750057 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
8.012
2.969
2.878
2.876
O
OCH3
N
S
88
10
9
8
7
6
5
4
3
2
1
-0
-1
ppm


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 29.8 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750571 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
99
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
ppm
2.37
1.92
9.63
1.95
3.53


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "port.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750571 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
99
ppm
8
7
6
5
4
3
2
1
12.19
9.90
09.84
10.06
18.21

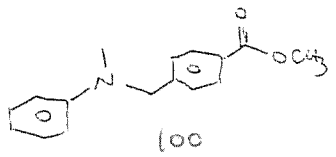

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0751019 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 8 sec
CH3
CH2
O
O
O
OCH3
100
10
9
8
7
6
5
4
3
2
1
-0
ppm


(scale up)
STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0751019 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
100
8
7
6
5
4
3
2
1
0
ppm
0.23
1.65
0.94
1.56
1.58
2.38
0.06
0.24
1.62
3.00
2.41
0.14
0.20

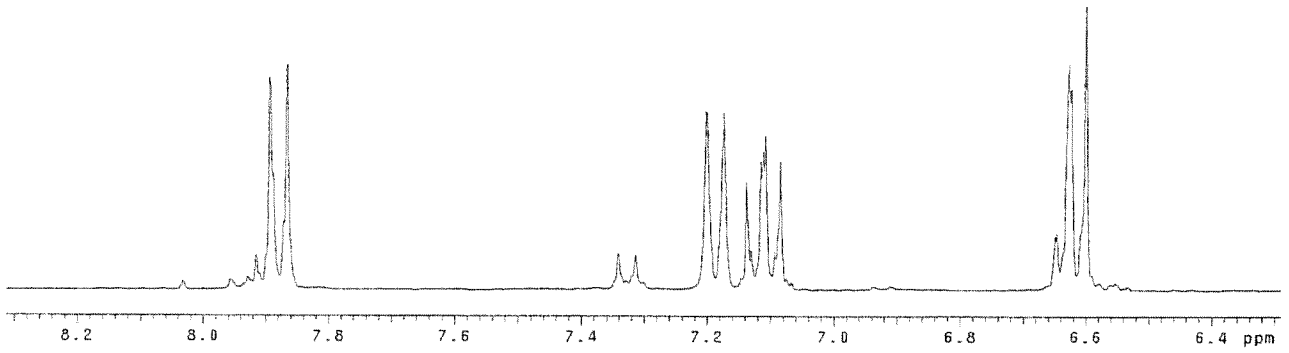

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.6 Hz
16 repetitions
OBSERVE H1, 300.0751019 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
100
7.916
7.894
7.888
7.872
7.866
7.341
7.313
7.283
7.261
7.174
7.139
7.130
7.116
7.111
7.108
7.094
7.085
6.850
6.646
6.628
6.623
6.600
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4 ppm

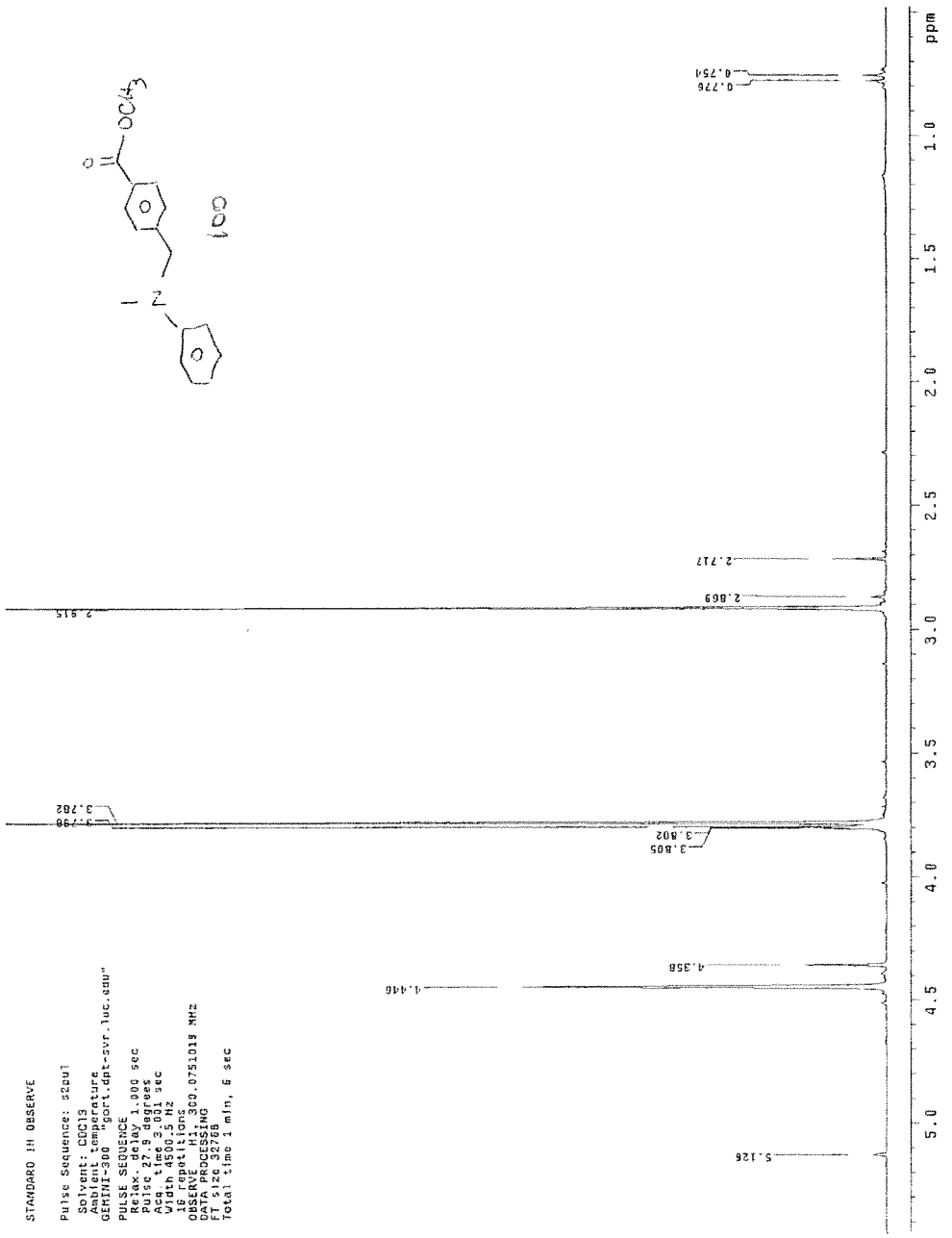
STANDARD IN OBSERVE

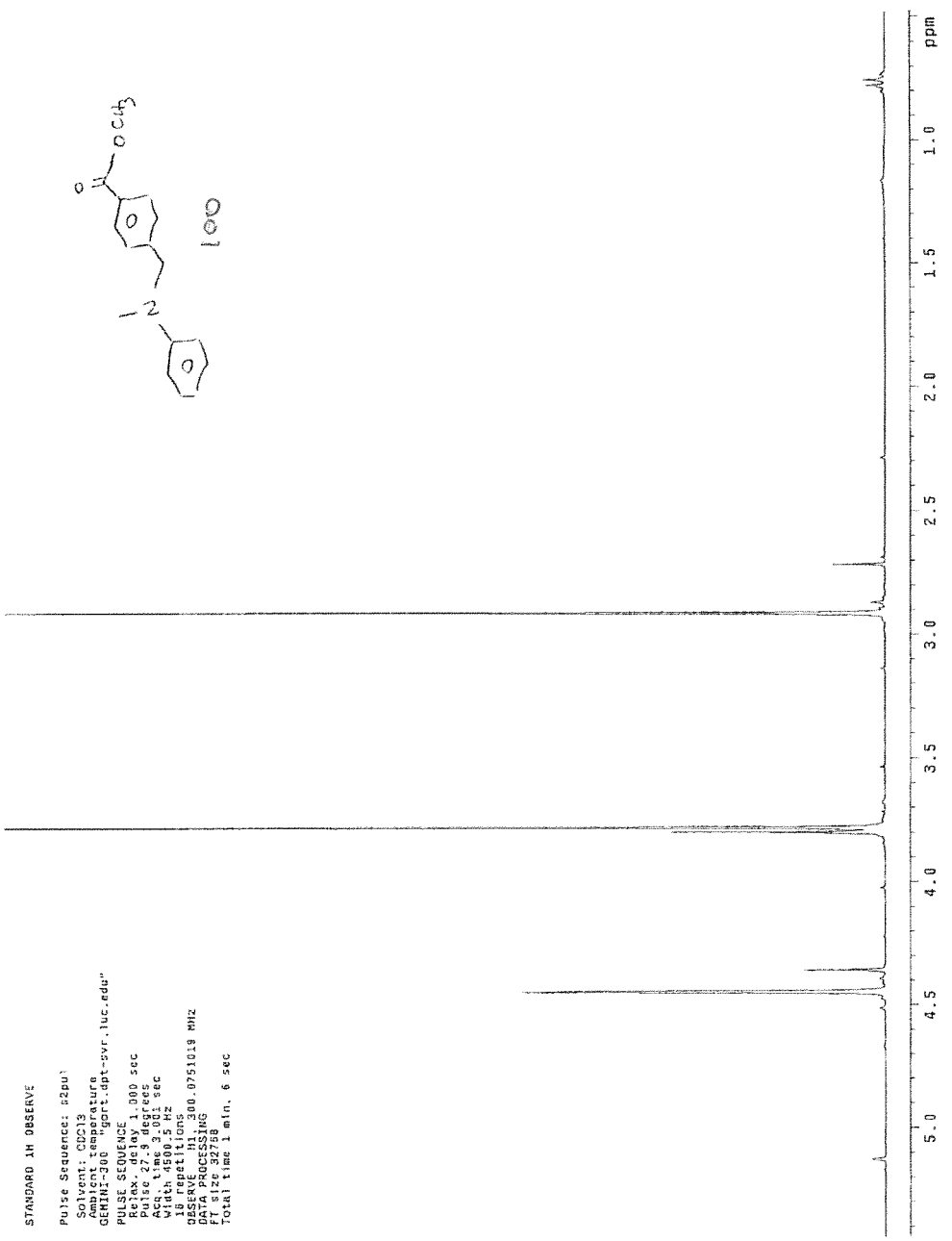
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0751019 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec



100

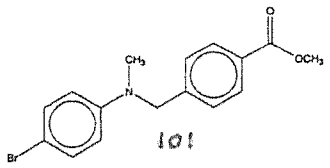



STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0751019 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
7.260
5.126
4.358
4.446
3.805
3.802
3.798
3.782
2.915
2.869
2.717
0.776
0.754
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-cvr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0751019 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
O
OCH3
1
2
100
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

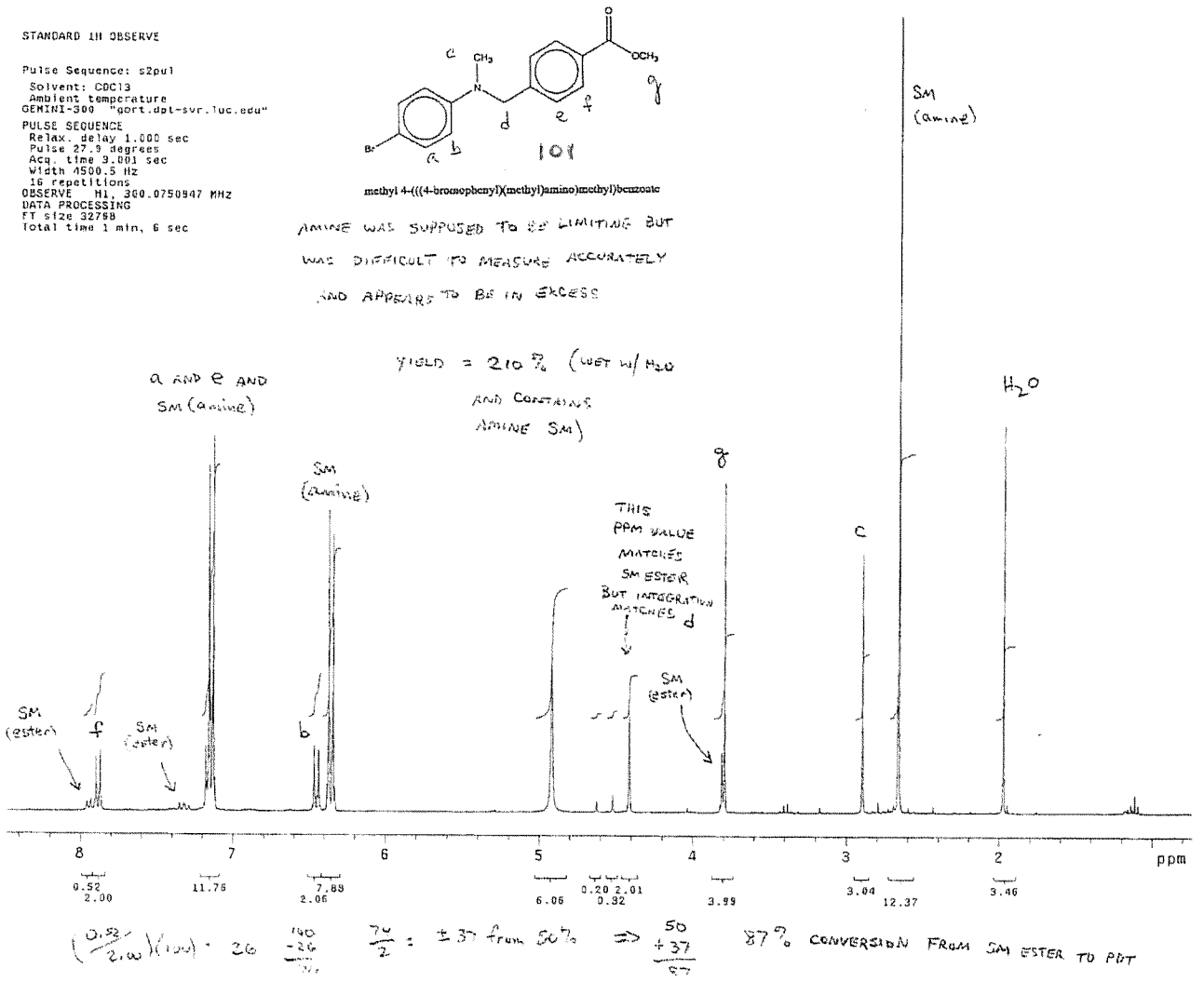
STANDARD 1H OBSERVE

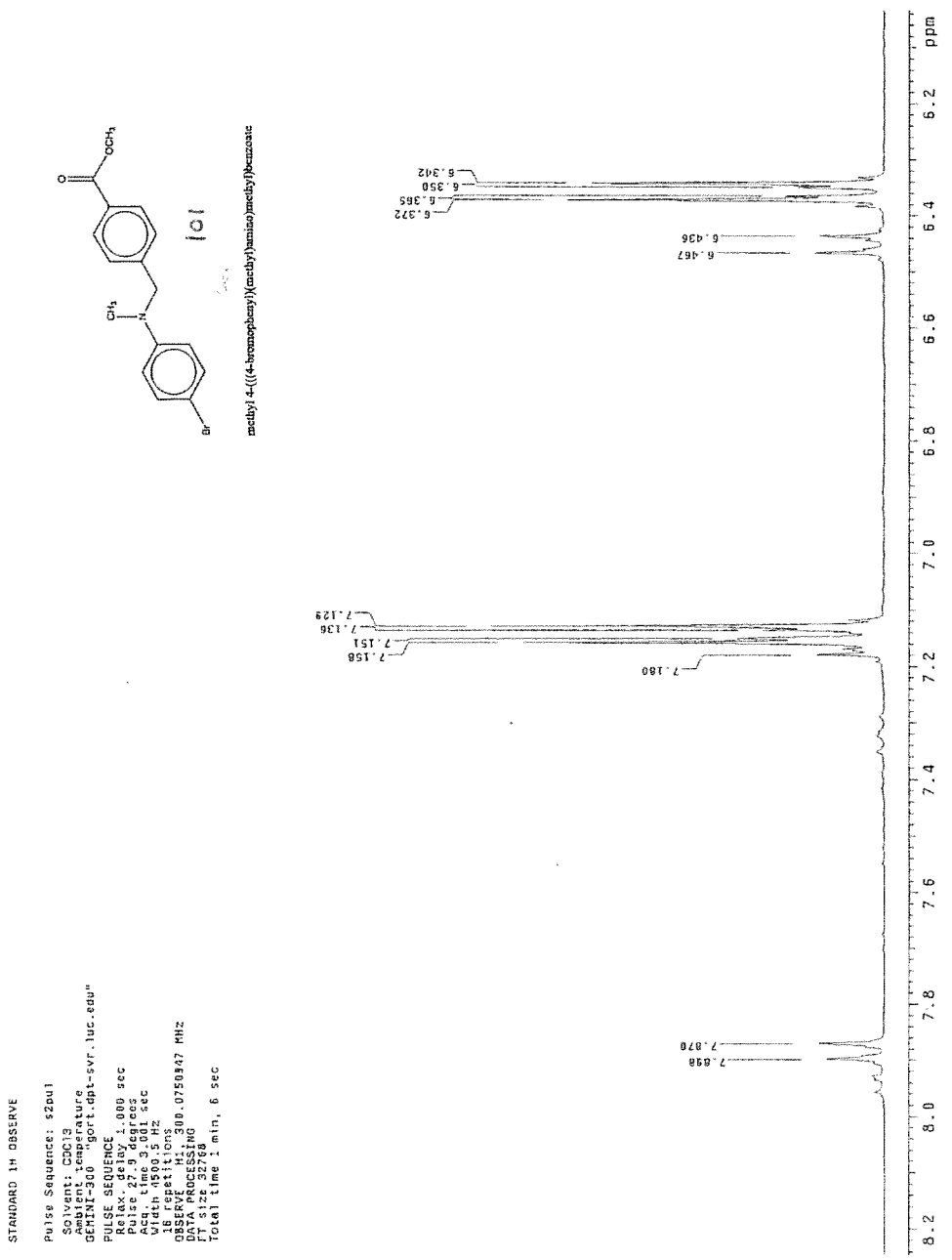
Pulse Sequence: s2pul
 Solvent: CDCl3
 Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
 Relax. delay 1.000 sec
 Pulse 27.9 degrees
 Acq. time 3.001 sec
 Width 4500.5 Hz
 16 repetitions
OBSERVE H1, 300.0750947 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 0 sec

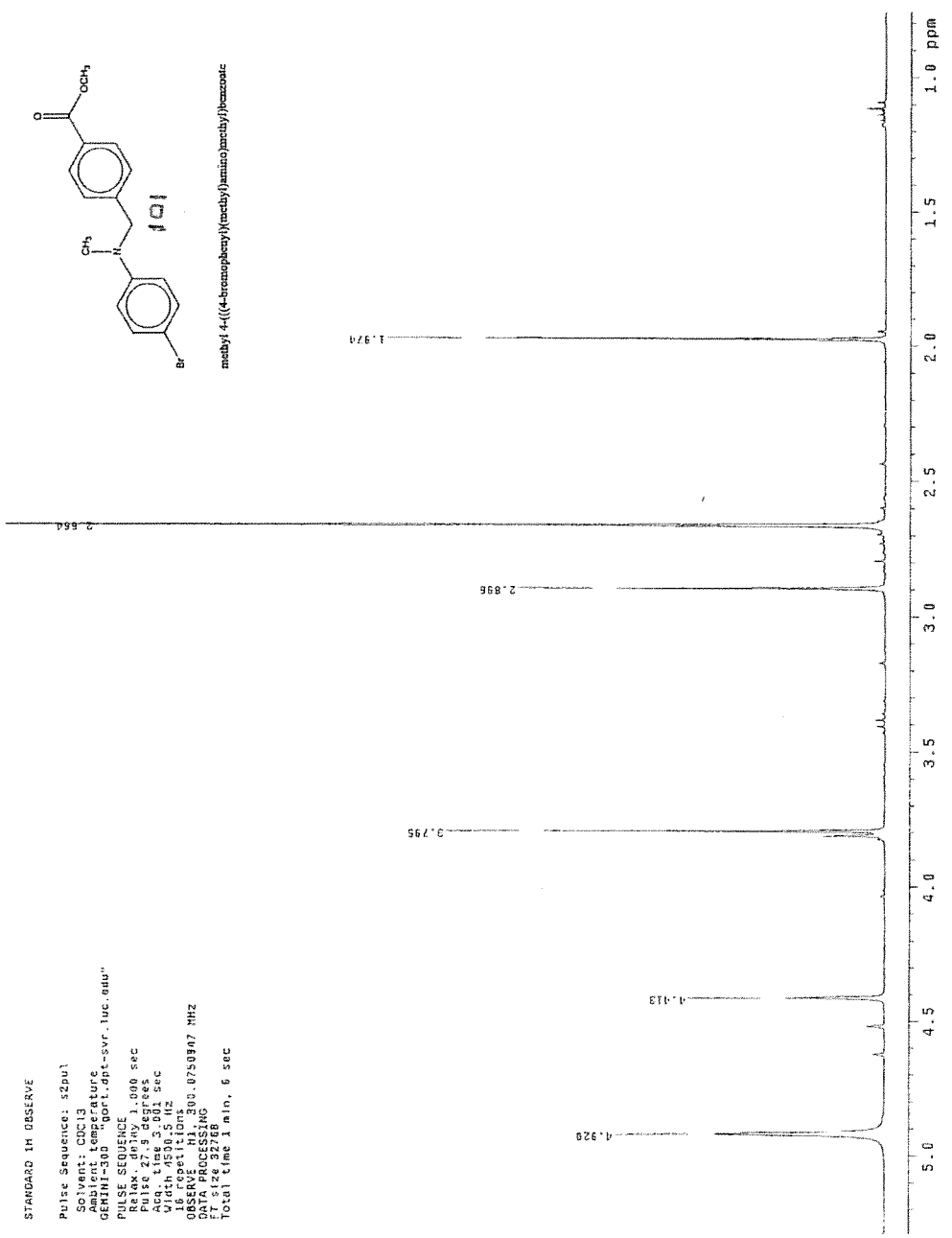


101

methyl 4-(((4-bromophenyl)(methyl)amino)methyl)benzoate

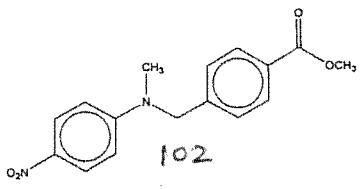

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "qort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750947 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
methyl 4-(((4-bromophenyl)(methyl)amino)methyl)benzoate
101
AMINE WAS SUPPOSED TO BE LIMITING BUT
WAS DIFFICULT TO MEASURE ACCURATELY
AND APPEARS TO BE IN EXCESS
YIELD = 210 % (WET W/ H2O
AND CONTAINS
AMINE SM)
a AND e AND
SM (amine)
SM
(amine)
THIS
PPM VALUE
MATCHES
SM ESTER
BUT INTEGRATION
MATCHES
SM
(ester)
SM
(ester)
SM
(ester)
SM
(amine)
H2O
ppm
0.52
2.00
11.76
7.89
2.06
6.06
0.20
0.32
2.01
3.99
3.04
12.37
3.46
(0.52/2.00)(100) = 26
140
-26
114
74/2 = ±37 from 50 % => 50 +37 87
87 % CONVERSION FROM SM ESTER TO PDT


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750847 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
101
methyl 4-(((4-bromophenyl)(methyl)amino)methyl)benzoate
7.878
7.858
7.180
7.158
7.151
7.136
7.129
6.467
6.436
6.372
6.365
6.350
6.342
8.2
8.0
7.8
7.6
7.4
7.2
7.0
6.8
6.6
6.4
6.2
ppm


methyl 4-(((4-bromophenyl)(methyl)amino)methyl)benzoate
101
STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4530.5 Hz
1 repetitions
OBSERVE H1, 300.0750907 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
2.664
1.924
2.896
3.795
1.913
4.928
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0 ppm

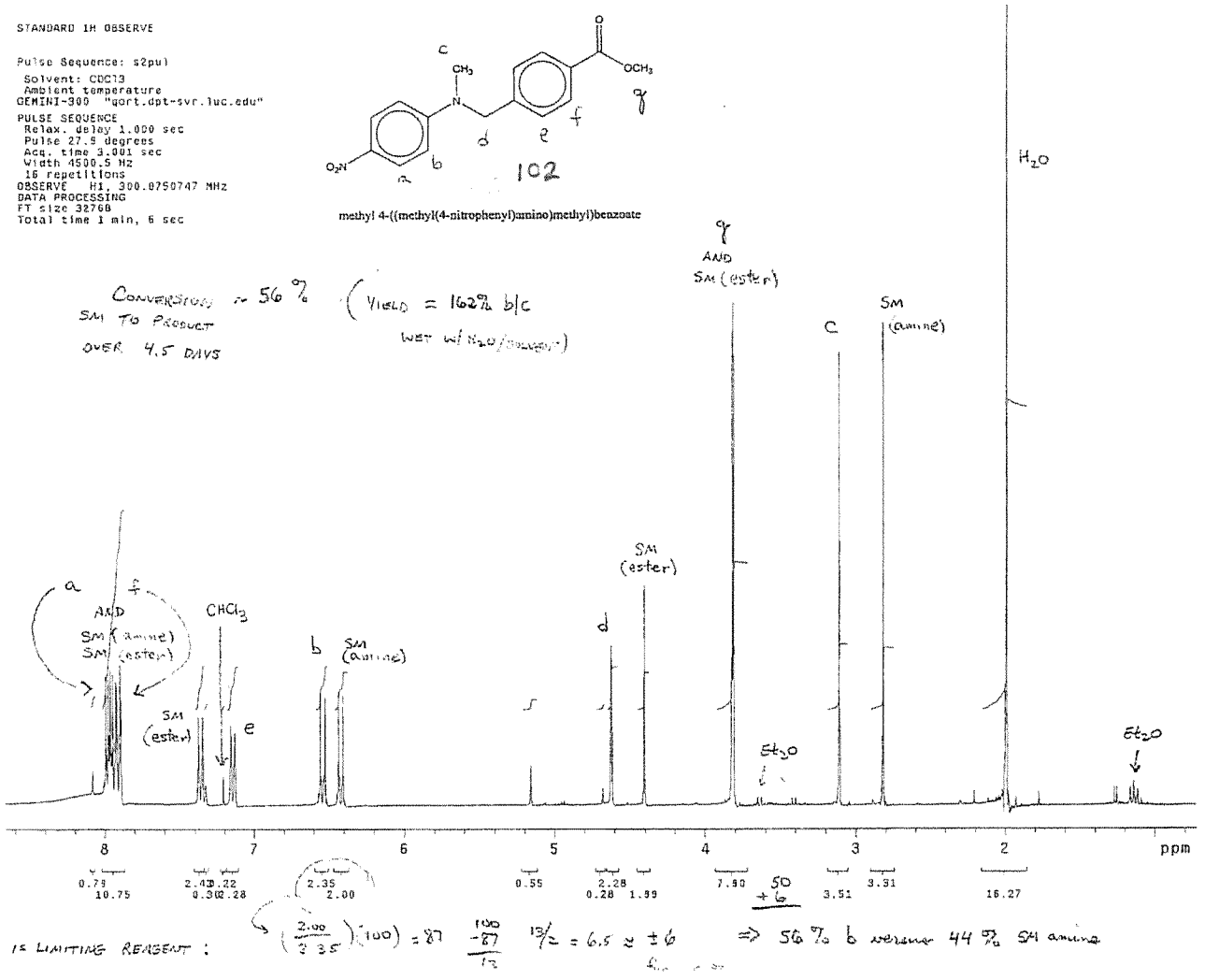
STANDARD 1H OBSERVE

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.8 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750747 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec



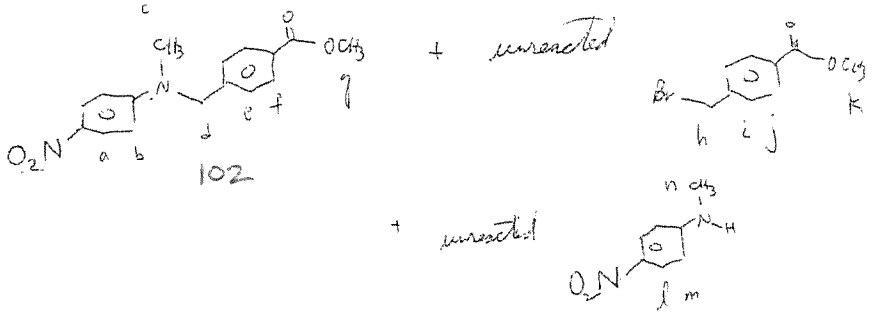
102

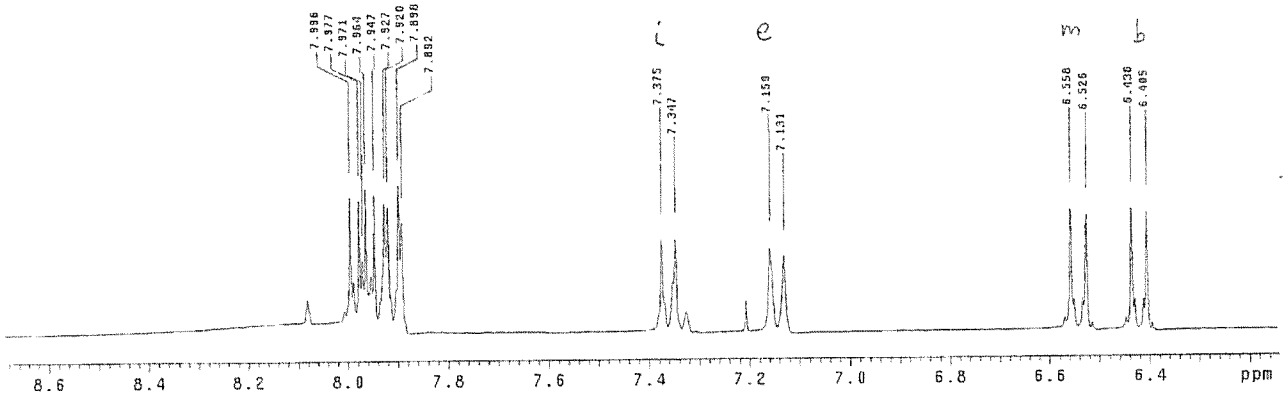
methyl 4-({methyl(4-nitrophenyl)amino}methyl)benzoate


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "qort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750747 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
102
methyl 4-((methyl(4-nitrophenyl)amino)methyl)benzoate
CONVERSION ~ 56 % (YIELD = 162% b/c
SM TO PRODUCT
OVER 4.5 DAYS
WET w/ H2O/solvent)
AND
SM (ester)
SM
(amine)
H2O
SM
(ester)
AND
SM (amine)
SM (ester)
CHCl3
SM
(amine)
Et2O
Et2O
ppm
0.79
10.75
2.43
0.30
3.22
2.28
2.35
2.00
0.55
2.28
0.28
1.99
7.90
50
+6
3.51
3.31
16.27
AMINE IS LIMITING REAGENT :
(2.00/3.35)(100) = 87
100
-87
13
13/2 = 6.5 ≈ ±6
=> 56 % b versus 44 % SM amine

STANDARD 1H OBSERVE

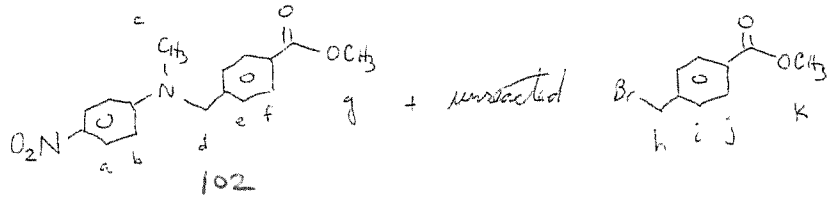
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750747 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec



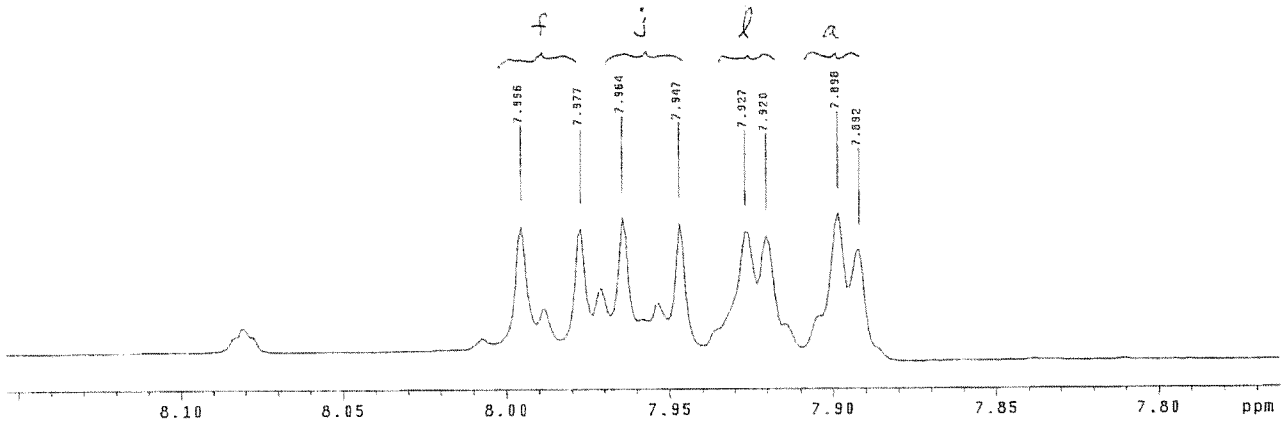


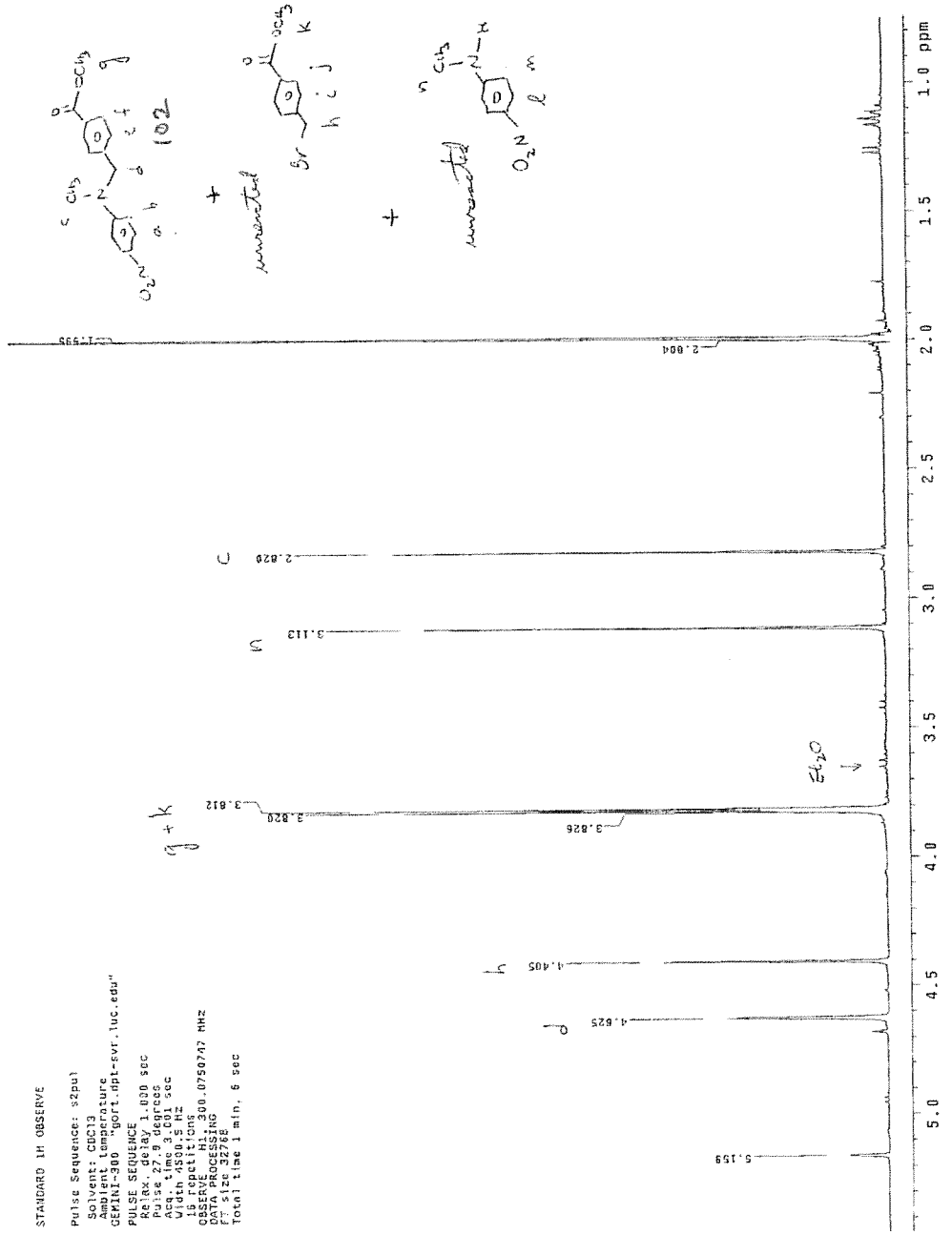
STANDARD 1H OBSERVE

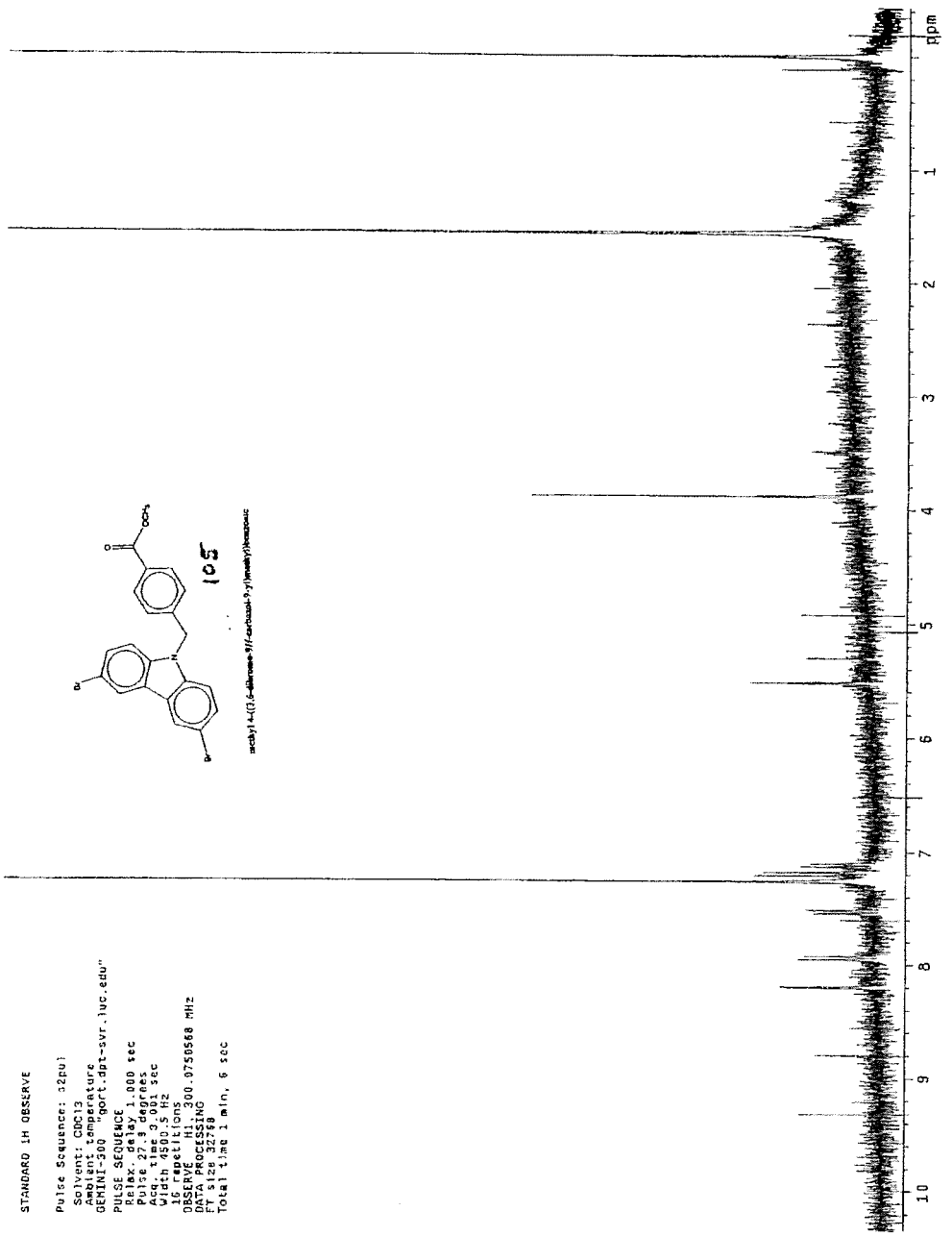
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750747 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec

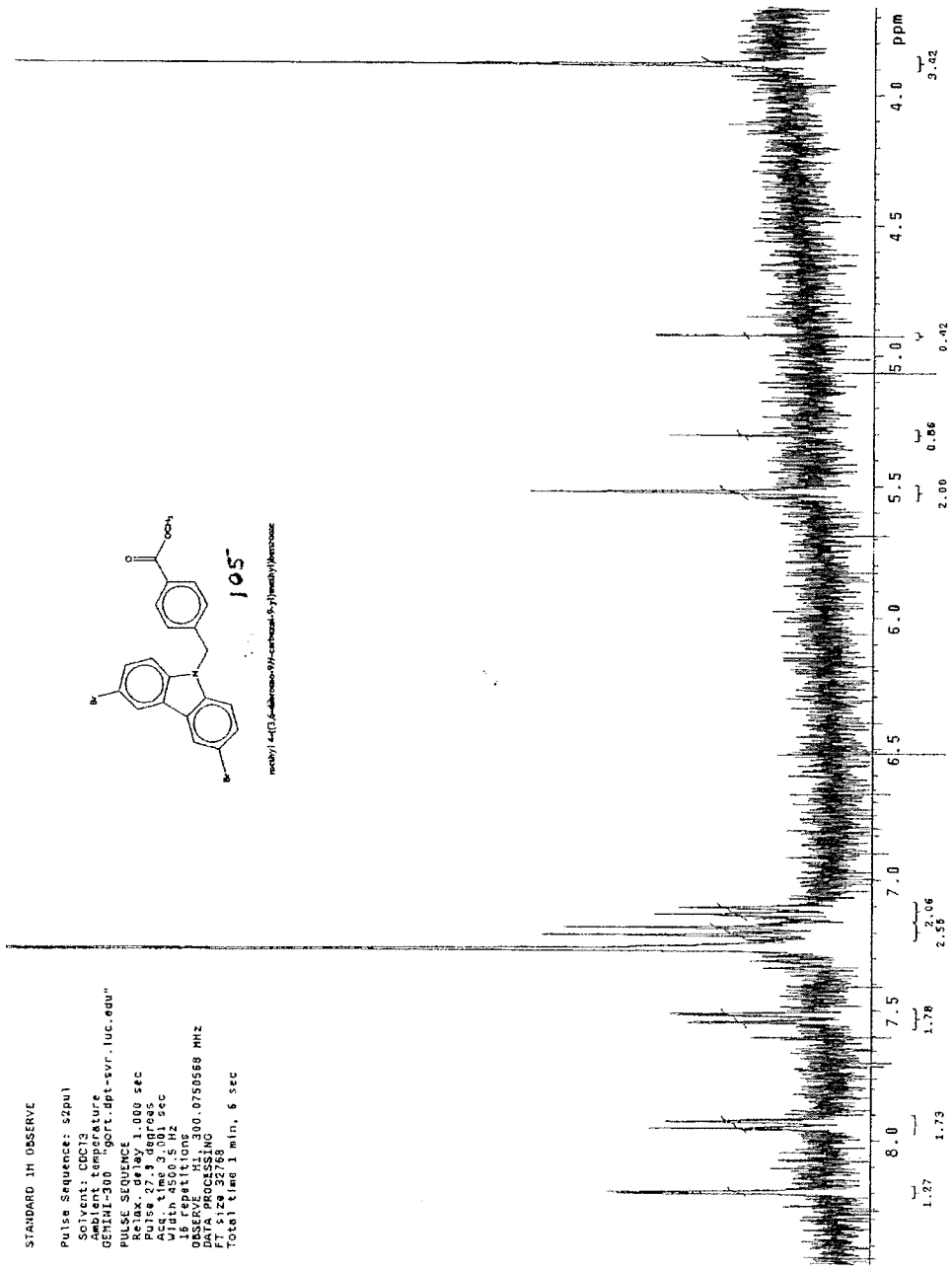


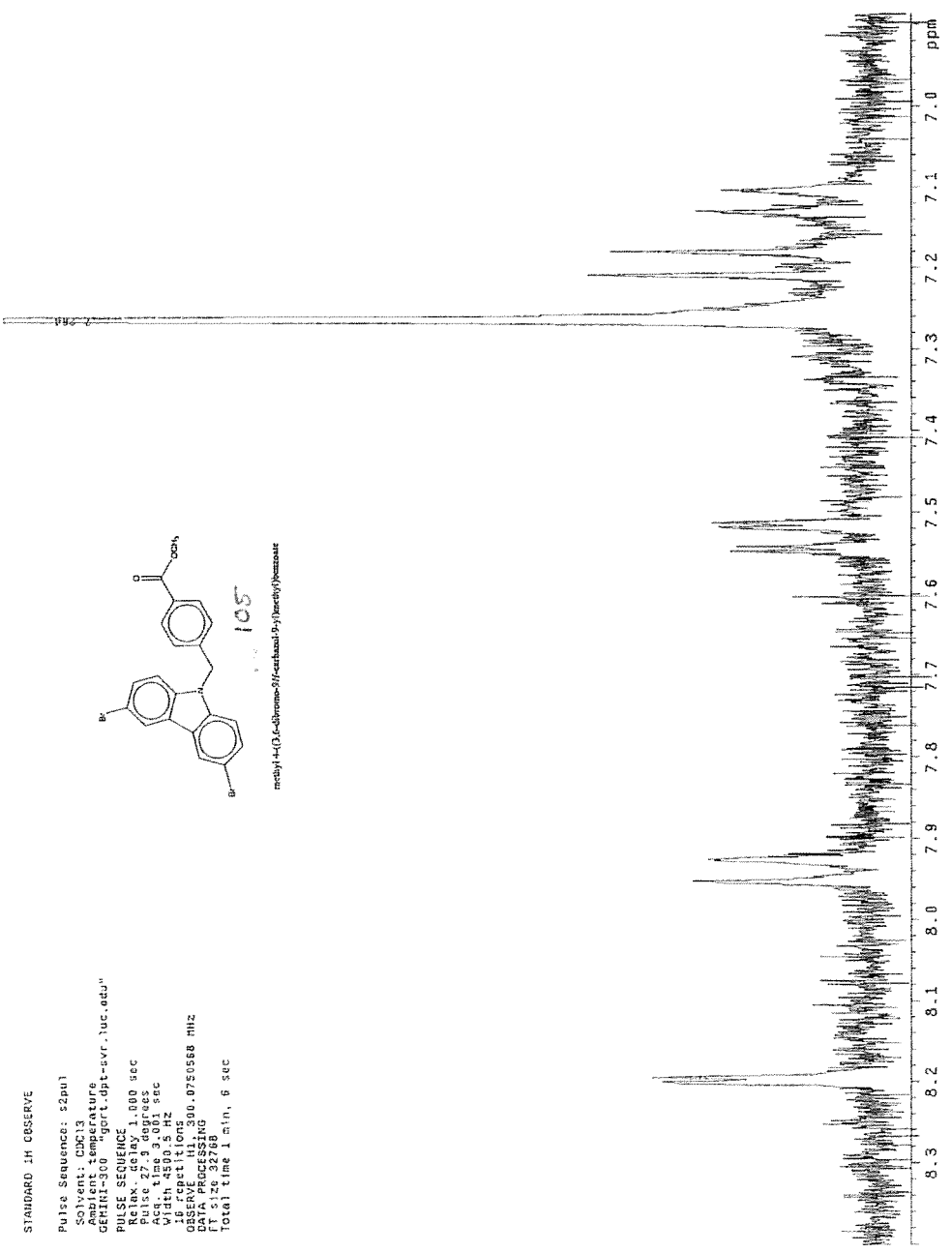
102




STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.8 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750747 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
5.159
4.625
4.405
3.826
3.820
3.812
3.113
2.829
2.804
1.595
H2O
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0 ppm

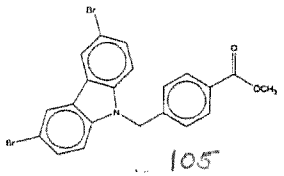

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750568 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
105
ppm


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.3 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750568 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
105
ppm
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
1.27
1.73
1.78
2.06
2.56
2.00
0.86
0.42
3.42


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-evr.luc.adu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 22.8 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750568 MHz
DATA PROCESSING
FT size 262768
Total time 1 min, 6 sec
Br
O
OCH3
Br
ios
methyl 4-((3,6-dibromo-9H-carbazol-9-yl)methyl)benzoate
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
ppm

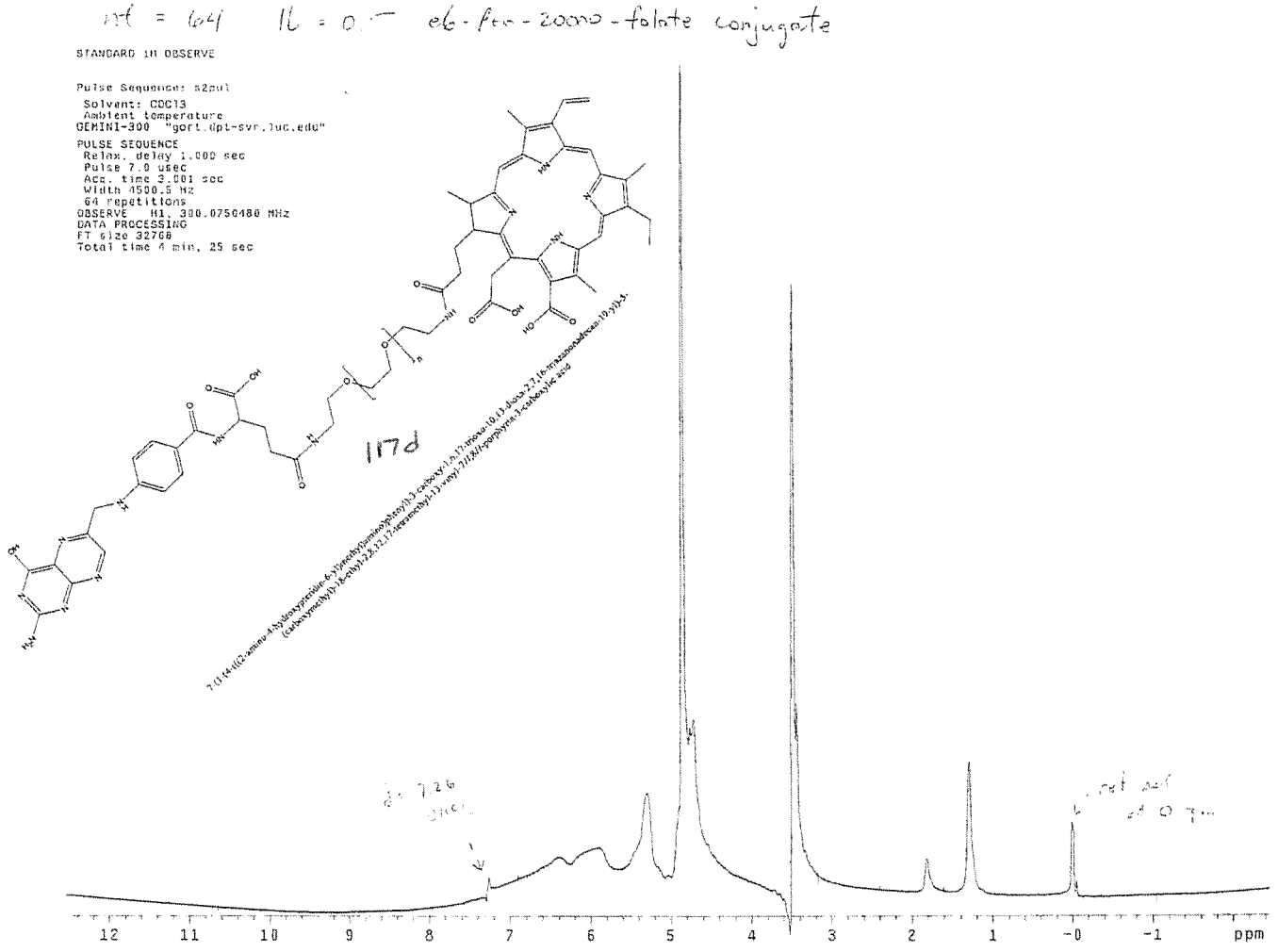
STANDARD 1H OBSERVE

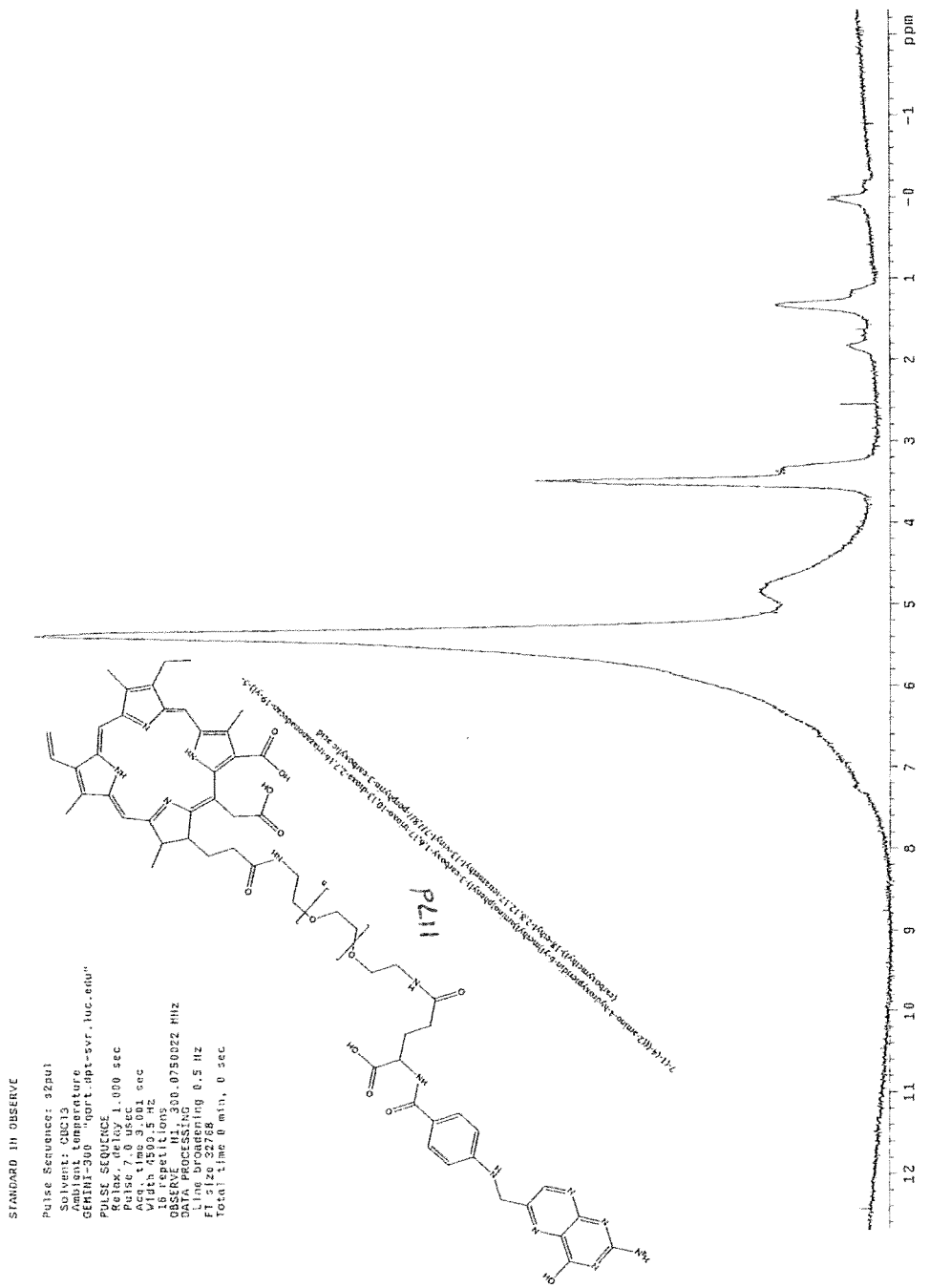
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750568 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec



105

methyl 4-[(3,6-dibromo-9H-carbazol-9-yl)methyl]benzoate


mf = 624 lL = 0.1 eb-fcu-2000-folate conjugate
STANDARD IN OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 7.0 usec
Acq. time 3.001 sec
Width 4500.5 Hz
64 repetitions
OBSERVE H1, 300.0750480 MHz
DATA PROCESSING
FT size 32768
Total time 4 min, 25 sec
117d
12
11
10
9
8
7
6
5
4
3
2
1
-0
-1
ppm


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "nport.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 7.0 usec
Acq. time 3.001 sec
Width 4500.5 Hz
1 repetitions
OBSERVE H1, 300.0750022 MHz
DATA PROCESSING
Line broadening 0.5 Hz
FT size 32768
Total time 0 min, 0 sec
11d
ppm

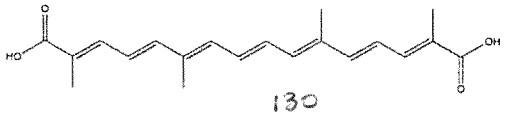
Anova 300 crocetin-base-saponification

STANDARD 1H OBSERVE

Archive directory: /export/home/vnmr1/vnmrsys/data
Sample directory:
File: PROTON

Pulse Sequence: s2pul
Solvent: DMSO
Temp. 25.0 C / 298.1 K
INOVA-300 "rodan.dpt-svr.luc.edu"

Relax. delay 1.000 sec
Pulse 30.0 degrees
Acq. time 3.740 sec
Width 4801.9 Hz
144 repetitions
OBSERVE H1, 299.7941030 MHz
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 40 min, 48 sec



130

(2*E*,4*E*,6*E*,8*E*,10*E*,12*E*,14*E*)-2,6,11,15-tetramethylhexadeca-2,4,6,8,10,12,14-heptaenedioic acid

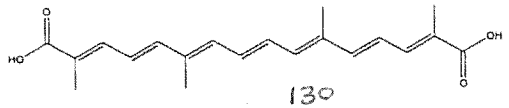
Reference to $CHCl_3$ (add 0.5 ppm to each δ value)

Anova 300 crocetin-base-saponification

STANDARD 1H OBSERVE

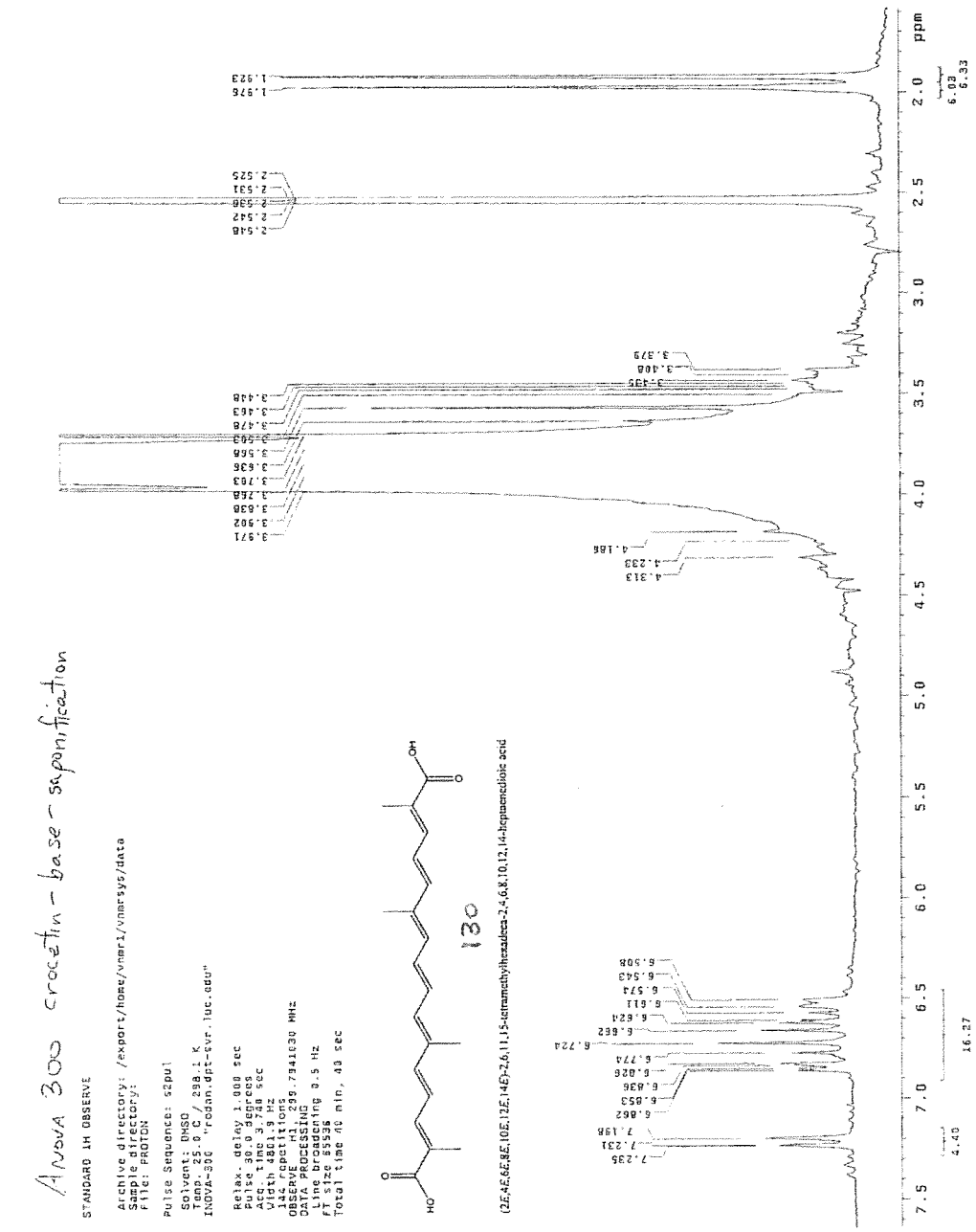
exp4 s2pul

SAMPLE		SPECIAL	
date	Jun 27 2014	temp	25.0
solvent	DMSO	gain	not used
file	exp	spin	20
ACQUISITION		hst	0.008
sw	4801.9	pw90	9.100
at	3.740	alfa	6.600
np	35918	FLAGS	
fb	3000	il	n
bs	16	in	n
ss	2	dp	y
d1	1.000	hs	nn
nt	512	PROCESSING	
ct	144	lb	0.50
TRANSMITTER		fn	not used
tn	H1	DISPLAY	
sfrq	299.795	sp	473.5
tof	299.5	wp	1817.3
tpwr	53	rf1	592.9
pw	3.038	rfp	0
DECOUPLER		rp	-80.9
dn	C13	lp	-14.9
dof	0	PLOT	
dm	nnn	wc	250
dmm	c	sc	0
dpwr	34	vs	20000
dmf	12345	th	12
		ai	ph



130

(2*E*,4*E*,6*E*,8*E*,10*E*,12*E*,14*E*)-2,6,11,15-tetramethylhexadeca-2,4,6,8,10,12,14-heptaenedioic acid


Nova 300 crocetin - base - saponification
STANDARD 1H OBSERVE
Archive directory: /export/home/vnmr1/vnmrsys/data
Sample directory:
Filter: PROTON
Pulse Sequence: s2pul
Solvent: DMSO
Temp. 25.0 C / 298.1 K
INOVA-300 "rodan.dpt-cvr.luc.edu"
Relax. delay 1.000 sec
Pulse 30.0 degrees
Acq. time 3.740 sec
Width 4801.9 Hz
8 repetitions
OBSERVE H1, 299.7941030 MHz
DATA PROCESSING
Line broadening 0.5 Hz
FT size 65536
Total time 40 min, 40 sec
OH
O
HO
O
130
(2E,4E,6E,8E,10E,12E,14E)-2,6,11,15-tetramethylhexadeca-2,4,6,8,10,12,14-heptaenedioic acid
1.923
1.976
2.525
2.531
2.538
2.542
2.548
3.371
3.902
3.638
3.758
3.783
3.636
3.566
3.478
3.463
3.448
3.379
3.408
3.435
4.188
4.233
4.313
6.508
6.543
6.574
6.611
6.624
6.662
6.724
6.774
6.826
6.836
6.853
6.862
7.198
7.231
7.235
ppm
6.08
6.33
4.40
16.27

Bis - β-D- ~~Crocetin~~ glucosyl(crocetin)

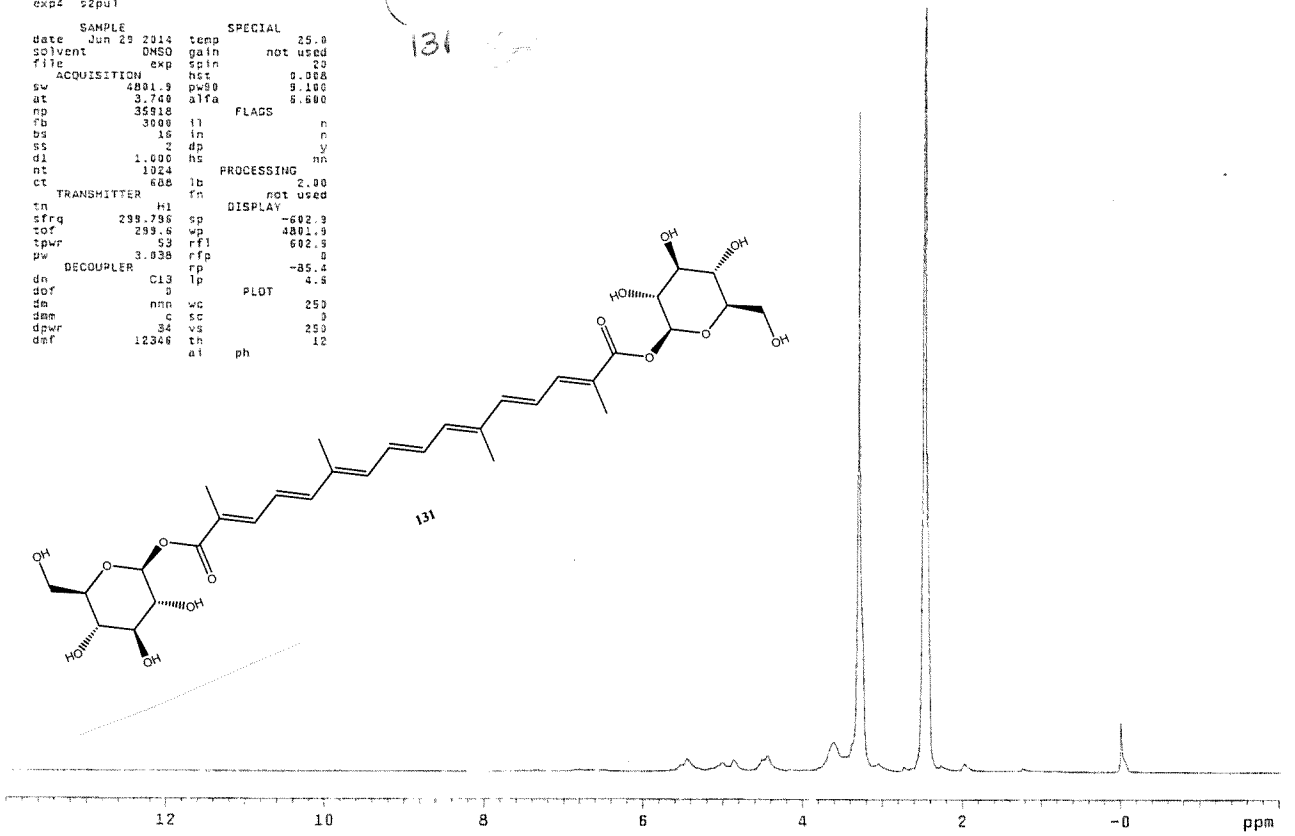
(REAGENT)

131

STANDARD 1H OBSERVE

exp4 s2pul

SAMPLE		SPECIAL	
date	Jun 25 2014	temp	25.0
solvent	DMSO	gain	not used
file	exp	spin	20
ACQUISITION		hst	0.008
sw	4801.9	pw90	9.100
at	3.740	alfa	5.600
np	35918	FLAGS	
fb	3000	il	n
bs	16	in	n
ss	2	dp	y
dl	1.000	hs	nn
nt	1024	PROCESSING	
ct	608	lb	2.00
TRANSMITTER		fn	not used
tn	H1	DISPLAY	
sfrq	299.796	sp	-602.9
tof	299.6	wp	4801.9
tpwr	59	rf1	602.5
pw	3.038	rfp	0
DECOUPLER		rp	-85.4
dn	C13	lp	4.5
dof	0	PLOT	
dm	nnn	wc	250
dmm	c	sc	0
dpwr	34	vs	250
dmf	12346	th	12
		ai	ph



Bis - β-D- glucosyl(rosetin)

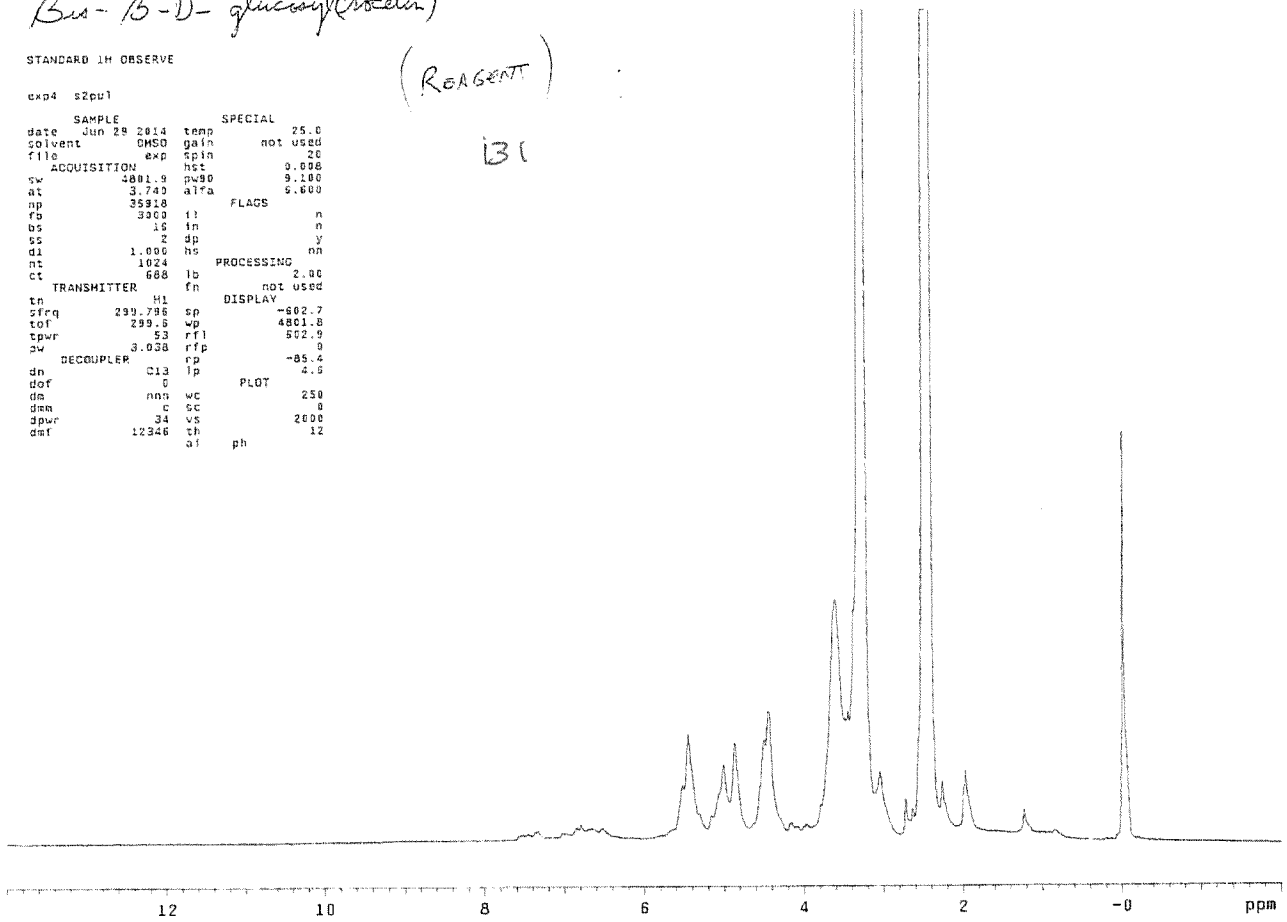
(REAGENT)

13(

STANDARD 1H OBSERVE

exp4 s2pul

SAMPLE		SPECIAL	
date	Jun 29 2014	temp	25.0
solvent	DMSO	gain	not used
file	exp	spin	20
ACQUISITION		hst	9.008
sw	4801.9	pw90	9.100
at	3.740	alfa	9.600
np	35918	FLAGS	
fb	3300	il	n
bs	16	in	n
ss	2	dp	y
d1	1.000	hs	nn
nt	1024	PROCESSING	
ct	688	lb	2.00
TRANSMITTER		fn	not used
tn	H1	DISPLAY	
sfrq	299.796	sp	-602.7
tof	299.6	wp	4801.8
tpwr	53	rfl	502.9
pw	3.038	rfp	0
DECOUPLER		rp	-85.4
dn	C13	lp	4.6
dof	0	PLOT	
dm	nnn	wc	250
dmm	c	sc	0
dpwr	34	vs	2800
dmf	12346	th	12
		ai	ph



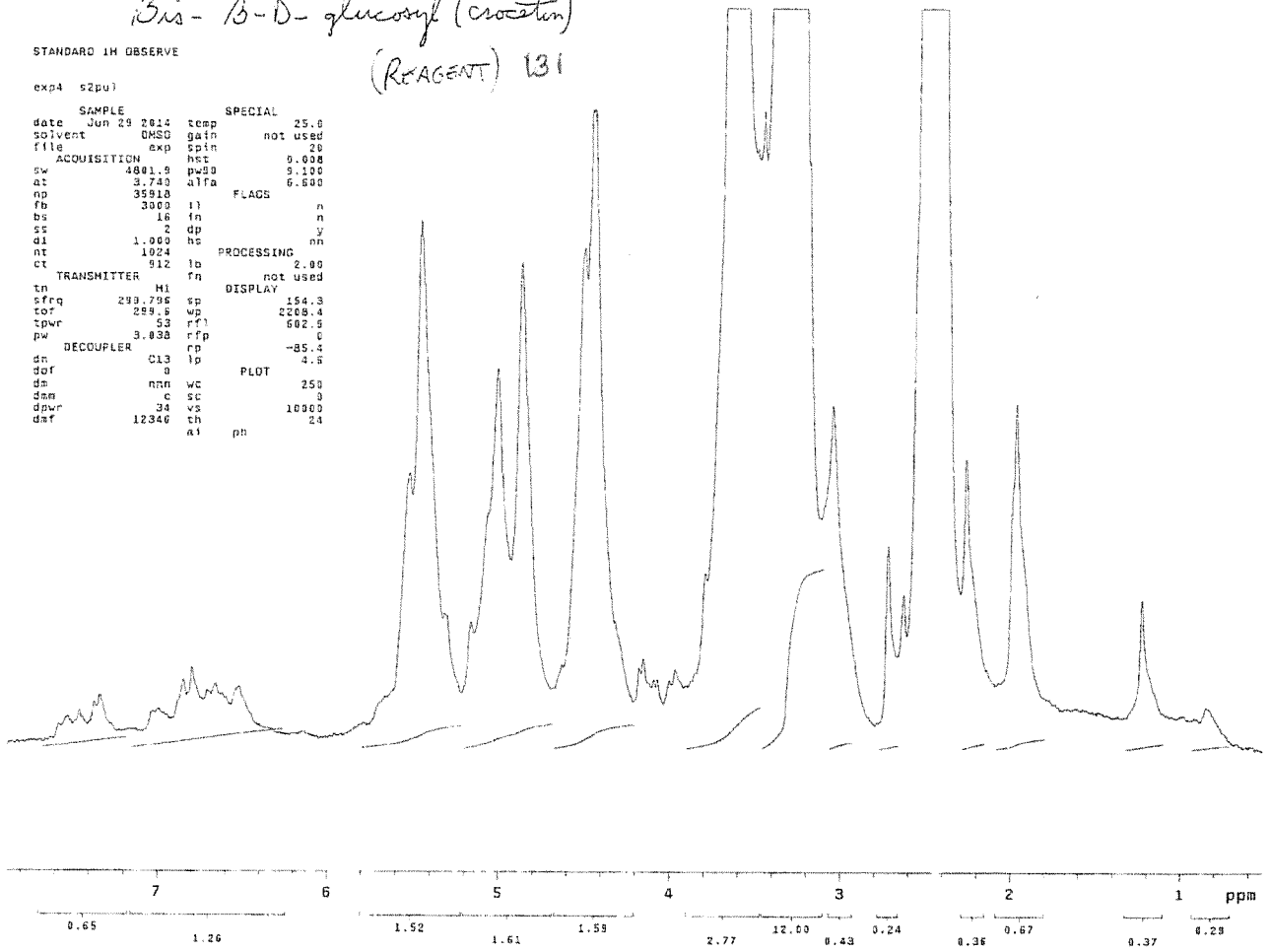
Bis - β-D- glucosyl (crocetin)

(REAGENT) 131

STANDARD 1H OBSERVE

exp4 s2pul

SAMPLE		SPECIAL	
date	Jun 29 2014	temp	25.0
solvent	DMSO	gain	not used
file	exp	spin	20
ACQUISITION		hst	0.008
sw	4801.9	pw90	9.100
at	3.740	alfa	6.600
np	35918	FLAGS	
fb	3000	il	n
bs	16	in	n
ss	2	dp	y
d1	1.000	hs	nn
nt	1024	PROCESSING	
ct	912	lb	2.00
TRANSMITTER		fn	not used
tn	H1	DISPLAY	
sfrq	299.796	sp	154.3
tof	299.6	wp	2208.4
tpwr	53	rfl	602.5
pw	3.038	rfp	0
DECOUPLER		rp	-85.4
dn	C13	lp	4.5
dof	0	PLOT	
dm	nnn	wc	250
dmm	c	sc	0
dpwr	34	vs	10000
dmf	12346	th	24
		ai	ph



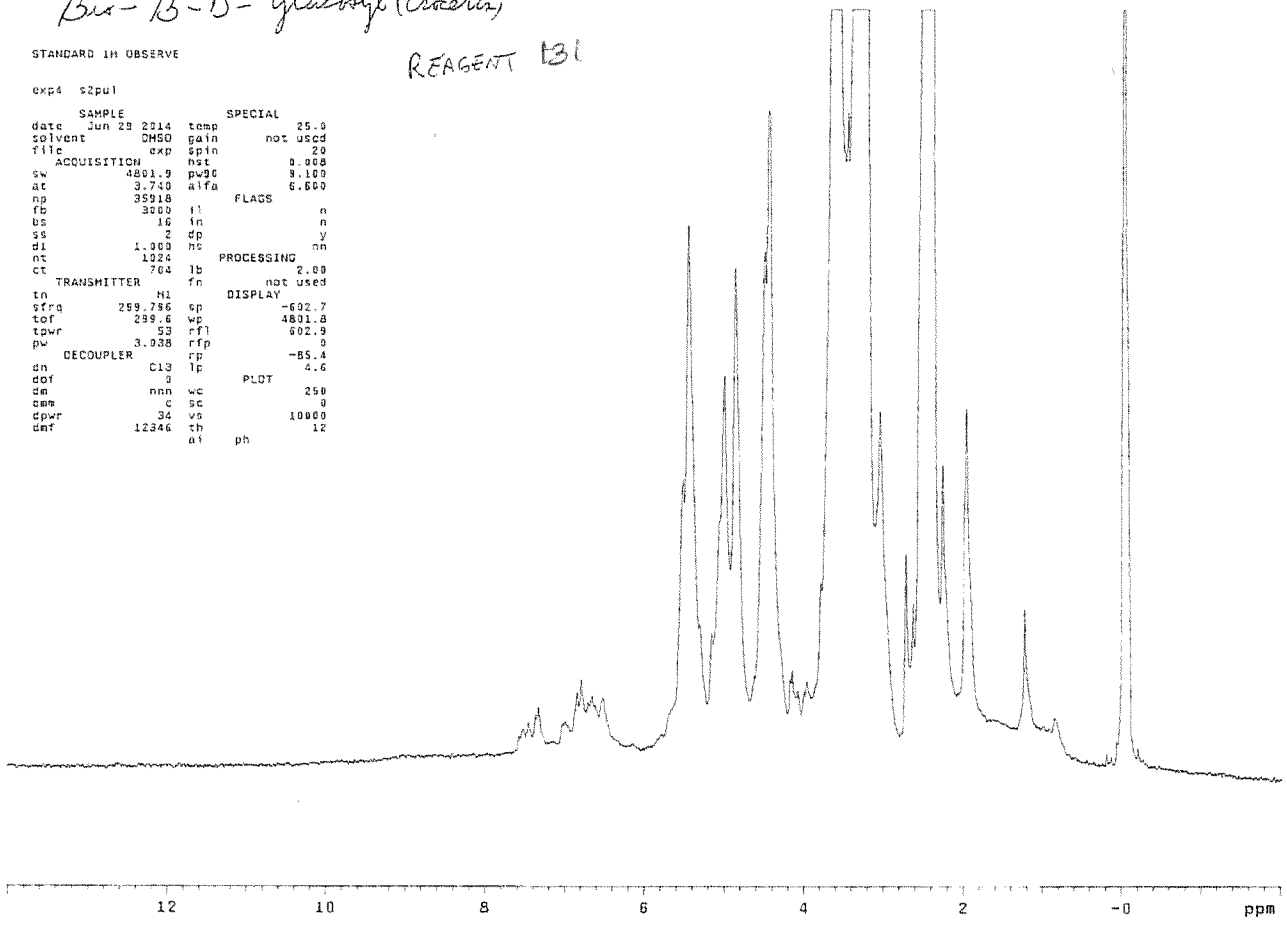
Bis-β-D-glucosyl(creatine)

REAGENT 131

STANDARD 1H OBSERVE

exp4 s2pul

SAMPLE			SPECIAL	
date	Jun 29 2014	temp		25.0
solvent	DMSO	gain		not used
file	exp	spin		20
ACQUISITION		hst		8.008
sw	4801.9	pw90		9.100
at	3.740	alfa		6.600
np	35918	FLAGS		
fb	3200	il		n
bs	16	in		n
ss	2	dp		y
d1	1.000	hs		nn
nt	1024	PROCESSING		
ct	704	lb		2.00
TRANSMITTER		fn		not used
tn	H1	DISPLAY		
sfrq	299.786	sp		-602.7
tof	299.6	wp		4801.8
tpwr	53	rfl		602.9
pw	3.038	rfp		0
DECOUPLER		rp		-85.4
dn	C13	lp		4.6
dof	0	PLOT		
dm	nnn	wc		250
dmm	c	sc		0
dpwr	34	vs		10000
dmf	12346	th		12
		ai	ph	



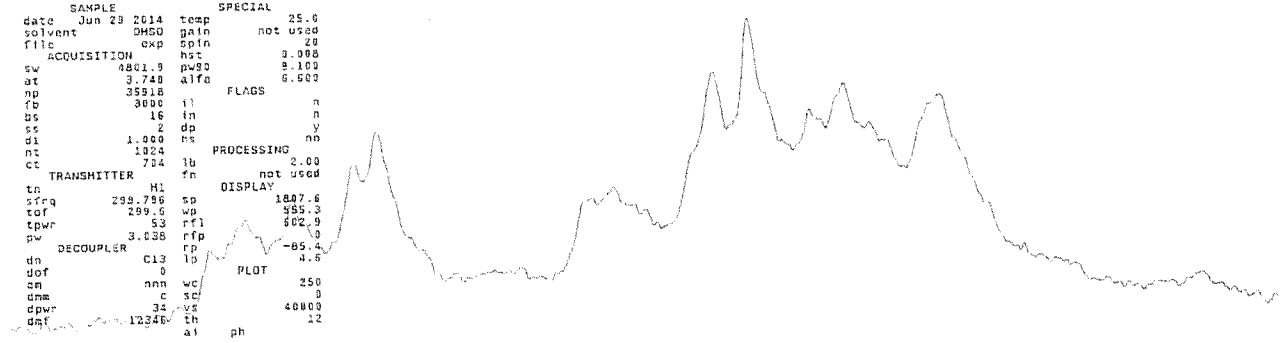
Bis - B-D - glucosyl(cyanidin)

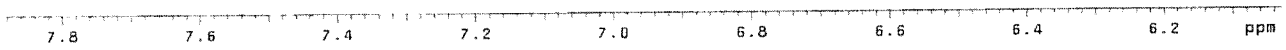
REAGENT 131

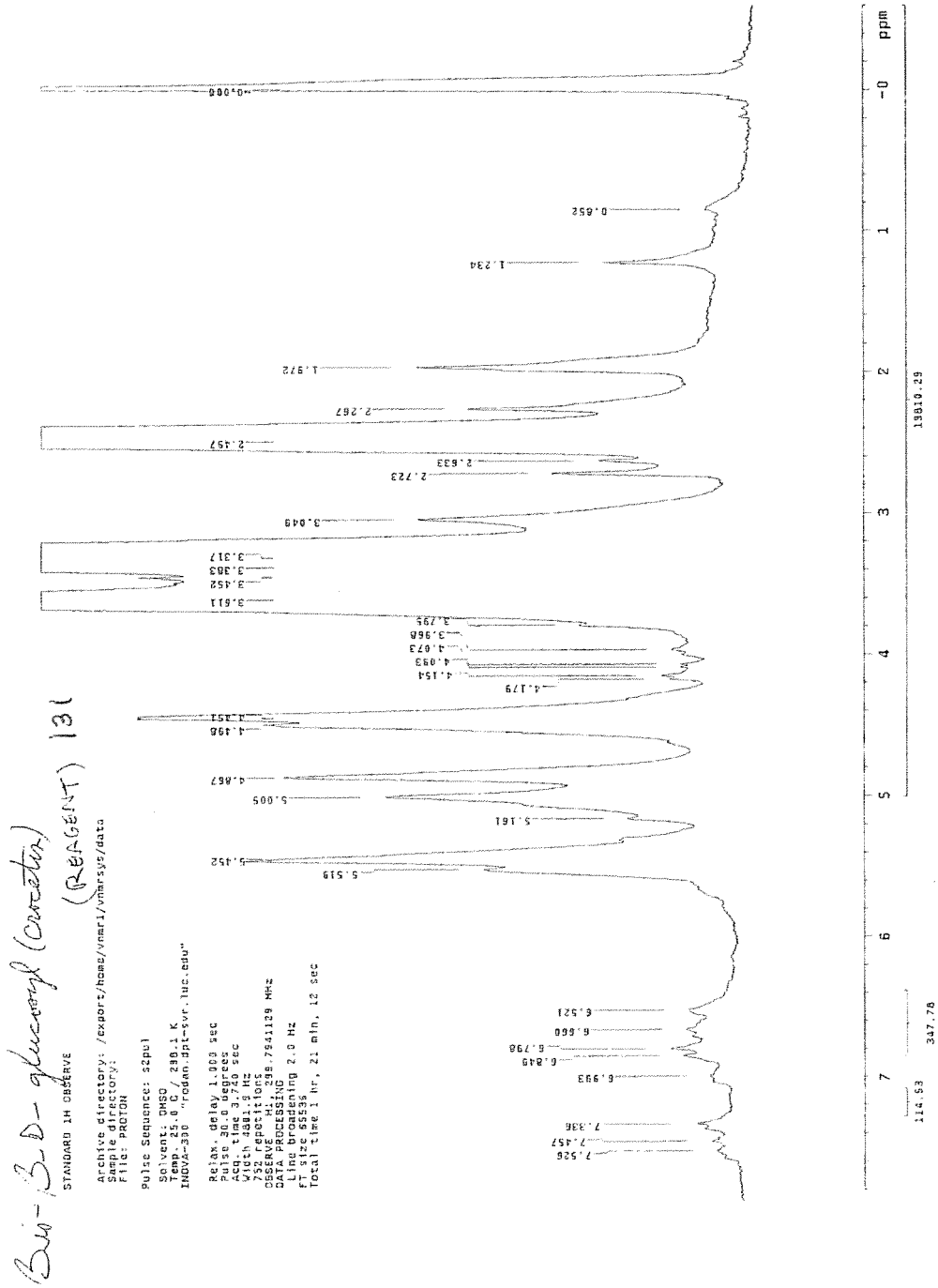
STANDARD 1H OBSERVE

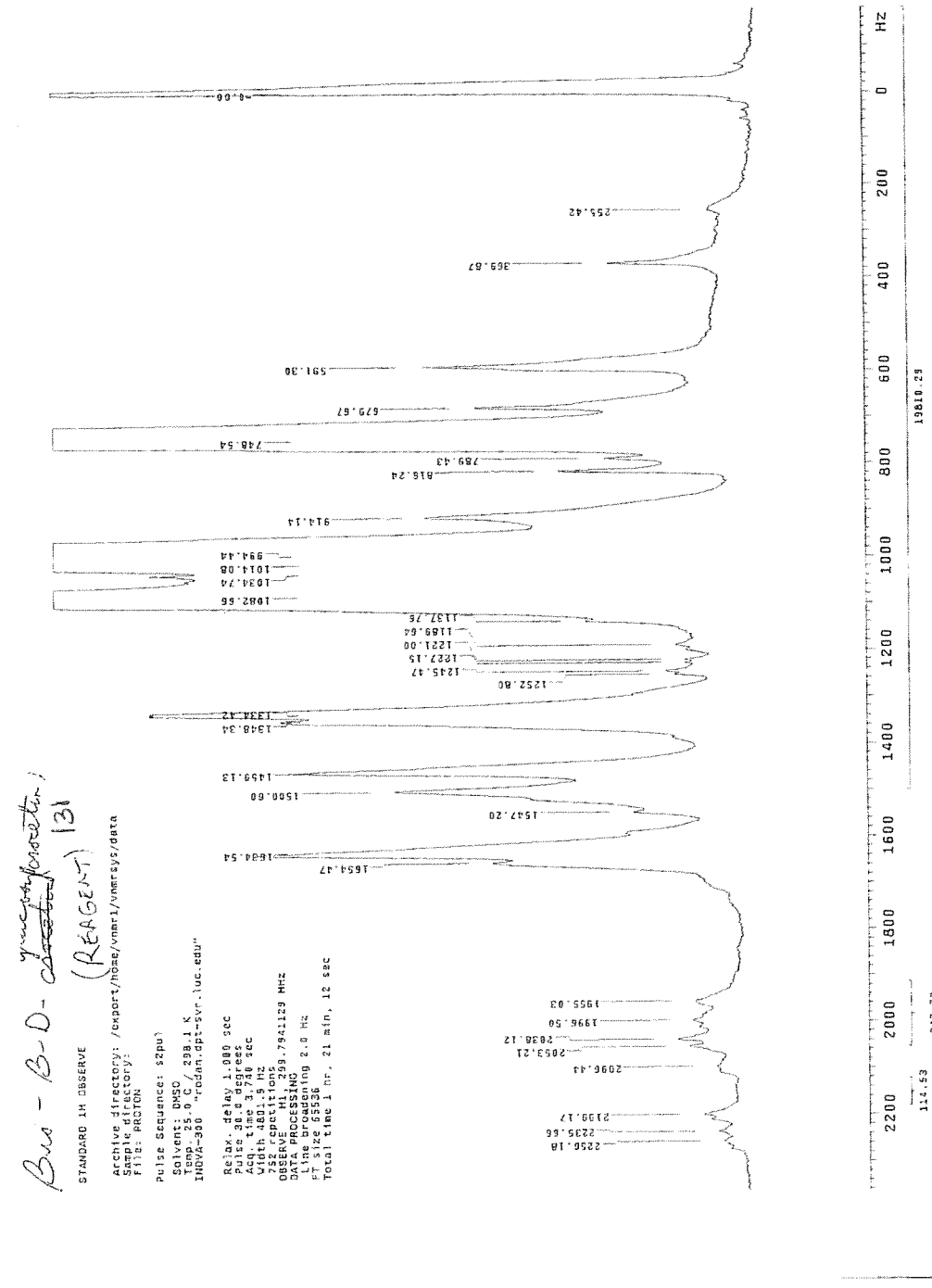
exp4 s2pul

SAMPLE		SPECIAL	
date	Jun 29 2014	temp	25.0
solvent	DMSO	gain	not used
file	exp	spin	20
ACQUISITION		hst	8.008
sw	4801.9	pw90	8.100
at	3.740	alfa	6.500
np	35918	**FLAGS**	
fb	3000	il	n
bs	16	in	n
ss	2	dp	y
d1	1.000	hs	nn
nt	1024	**PROCESSING**	
ct	704	lb	2.00
TRANSMITTER		fn	not used
tn	H1	**DISPLAY**	
sfrq	299.796	sp	1807.6
tof	299.6	wp	5855.3
tpwr	53	rfl	902.9
pw	3.038	rfp	0
DECOUPLER		rp	-85.4
dn	C13	lp	4.6
dof	0	**PLOT**	
dm	nnn	wc	250
dmm	c	sc	0
dpwr	34	vs	40800
dmf	12346	th	12
		ai	ph






Bis-3,5-D-glucuronyl (carotin)
(REAGENT) 131
STANDARD 1H OBSERVE
Archive directory: /export/home/vnmr1/vnmrsys/data
Sample directory:
File: PROTON
Pulse Sequence: s2pul
Solvent: DMSO
Temp. 25.0 C / 298.1 K
INOVA-300 "rodan.dpt-svr.luc.edu"
Relax. delay 1.000 sec
Pulse 30.0 degrees
Acq. time 3.740 sec
Width 4981.8 Hz
1 repetitions
OBSERVE H1, 299.7641129 MHz
DATA PROCESSING
Line broadening 2.0 Hz
FT size 65536
Total time 1 hr, 21 min, 12 sec
0.000
0.652
1.234
1.972
2.267
2.457
2.633
2.723
3.049
3.317
3.383
3.452
3.613
3.795
3.968
4.073
4.093
4.154
4.179
4.351
4.398
4.867
5.005
5.161
5.452
5.519
6.321
6.660
6.798
6.849
6.993
7.336
7.457
7.526
0
1
2
3
4
5
6
7
ppm
19819.29
114.53
347.78


Bun - B-D- glucopyranoside (REAGENT) 13
STANDARD 1H OBSERVE
Archive directory: /export/home/vmrl/vnmrsys/data
Sample directory:
File: ACETON
Pulse Sequence: s2pul
Solvent: DMSO
Temp. 25.0 C / 298.1 K
INOVA-300 "rodan.dpt-svr.luc.edu"
Relax. delay 1.000 sec
Pulse 30.0 degrees
Acq. time 3.740 sec
Width 4801.5 Hz
752 repetitions
OBSERVE H1, 299.7941129 MHz
DATA PROCESSING
Line broadening 2.0 Hz
FT size 65536
Total time 1 hr, 21 min, 12 sec
8.66
255.42
369.87
591.30
679.67
748.54
789.43
818.24
914.14
994.44
1014.08
1034.74
1082.66
1137.76
1189.64
1221.00
1227.15
1245.47
1252.80
1334.12
1348.34
1459.13
1500.60
1547.20
1654.47
1884.54
1955.03
1996.50
2038.12
2053.21
2096.44
2139.17
2235.66
2256.18
Hz
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
114.53
347.78
19810.29

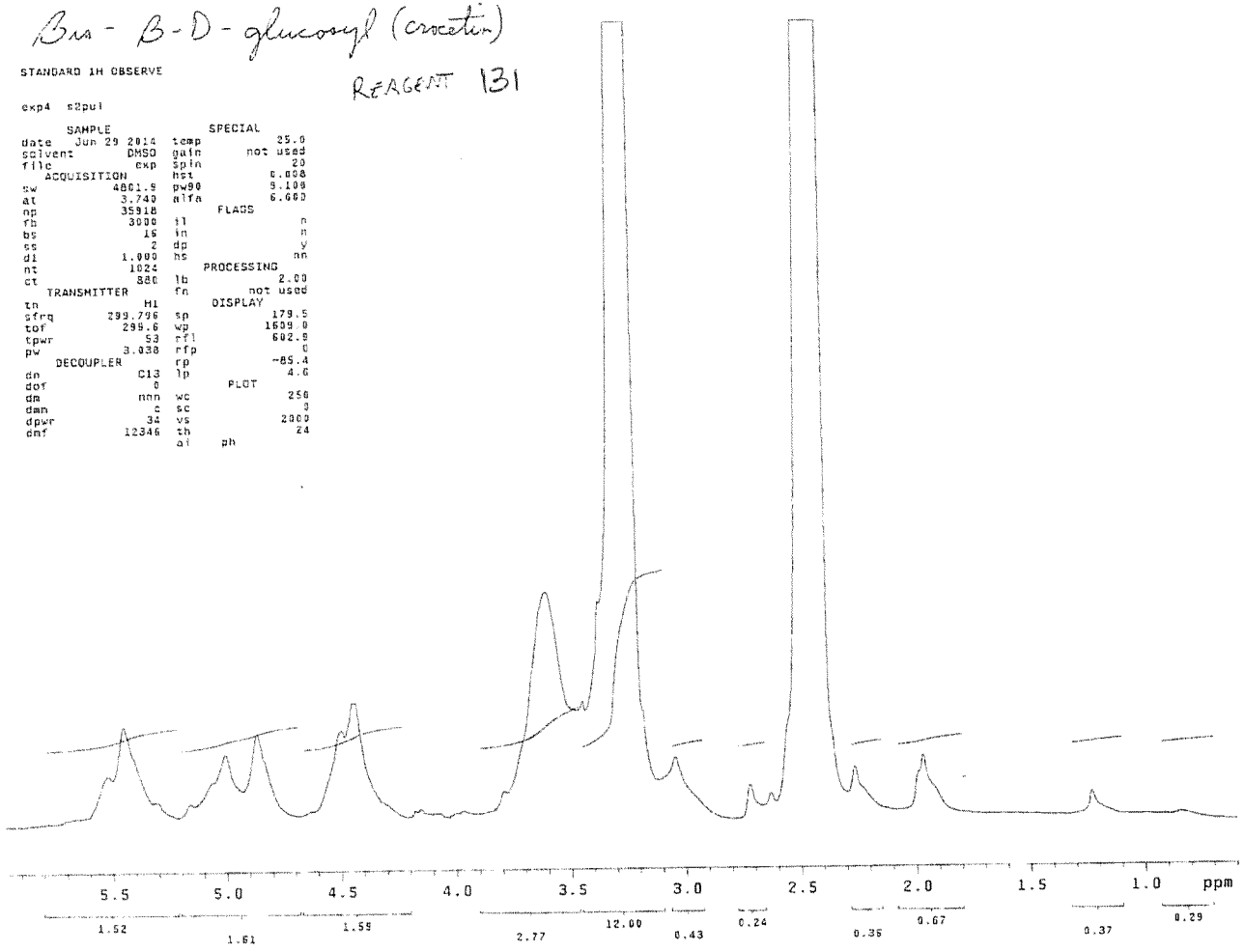
Bis - β-D-glucosyl (crocetin)

REAGENT 131

STANDARD 1H OBSERVE

exp4 s2pul

SAMPLE		SPECIAL	
date	Jun 29 2014	temp	35.0
solvent	DMSO	gain	not used
file	exp	spin	20
ACQUISITION		hst	0.008
sw	4861.9	pw90	9.100
at	3.740	alfa	6.660
np	35918	FLAGS	
fb	3000	il	n
bs	16	in	n
ss	2	dp	y
d1	1.000	hs	nn
nt	1024	PROCESSING	
ct	880	lb	2.00
TRANSMITTER		fn	not used
tn	H1	DISPLAY	
sfrq	299.796	sp	179.5
tof	299.6	wp	1609.0
tpwr	52	rfl	602.5
pw	8.038	rfp	0
DECOUPLER		rp	-85.4
dn	C13	lp	4.6
dof	0	PLOT	
dm	nnn	wc	250
dmm	c	sc	0
dpwr	34	vs	2800
cnf	12346	th	24
		at	ph



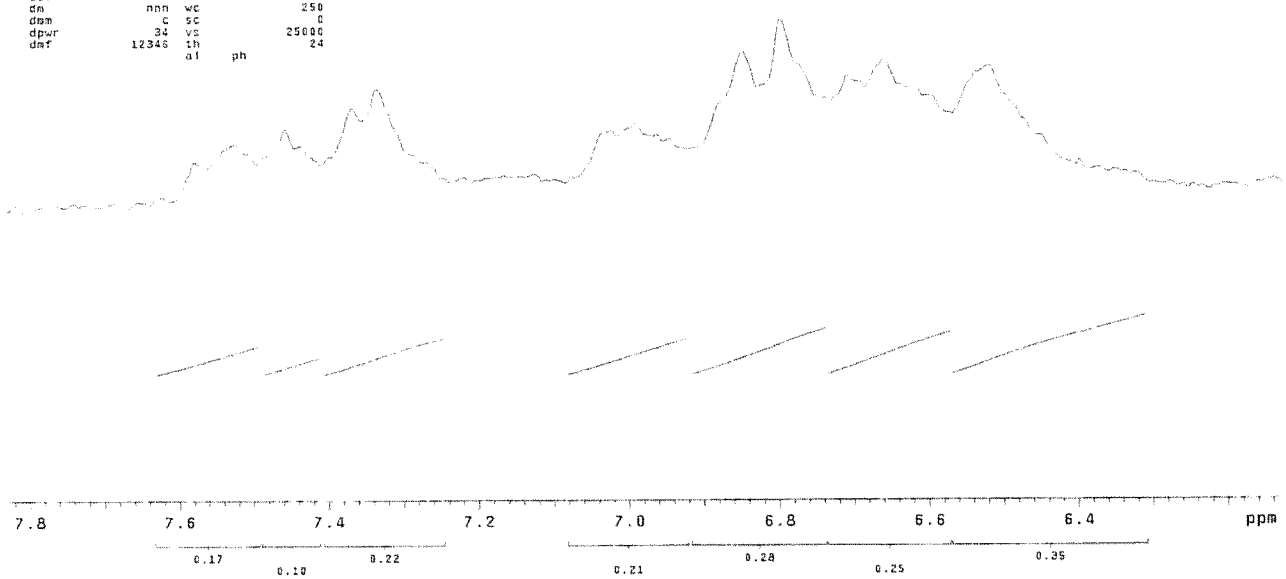
Bis - β-D-glucosyl (crocetin)

REAGENT 131

STANDARD 1H OBSERVE

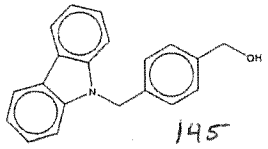
exp4 s2pul

SAMPLE		SPECIAL	
date	Jun 29 2014	temp	25.0
solvent	DMSO	gain	not used
file	exp	spin	20
ACQUISITION		hst	0.008
sw	4801.9	pw90	9.100
at	3.740	alfa	6.600
np	35918	FLAGS	
fb	3000	ll	n
bs	16	in	n
ss	2	dp	y
dl	1.000	hs	nn
nt	1024	PROCESSING	
ct	960	lb	2.00
TRANSMITTER		fn	not used
tn	H1	DISPLAY	
sfrq	299.796	sp	1837.4
tof	299.6	wp	5110.7
tpwr	53	rfl	602.9
pw	3.038	rfp	0
DECOUPLER		rp	-85.4
dn	C13	lp	4.6
dof	0	PLOT	
dm	nnn	wc	250
dmm	c	sc	0
dpwr	34	vs	25000
dmf	12346	th	24
		al	ph



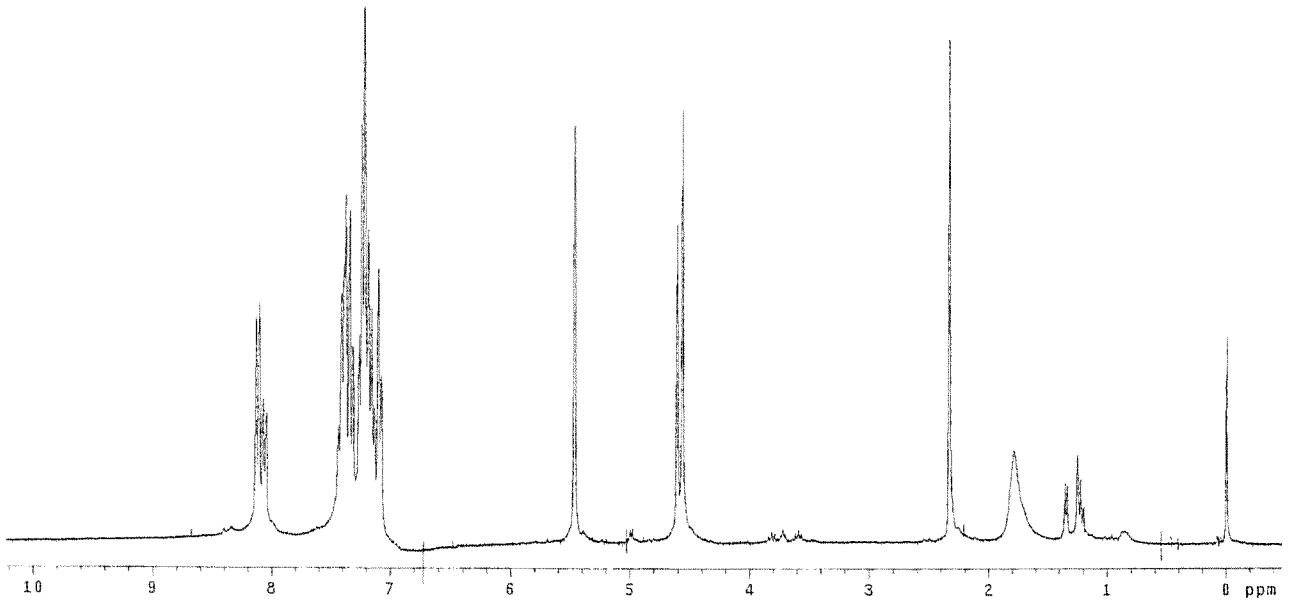
STANDARD 1H OBSERVE

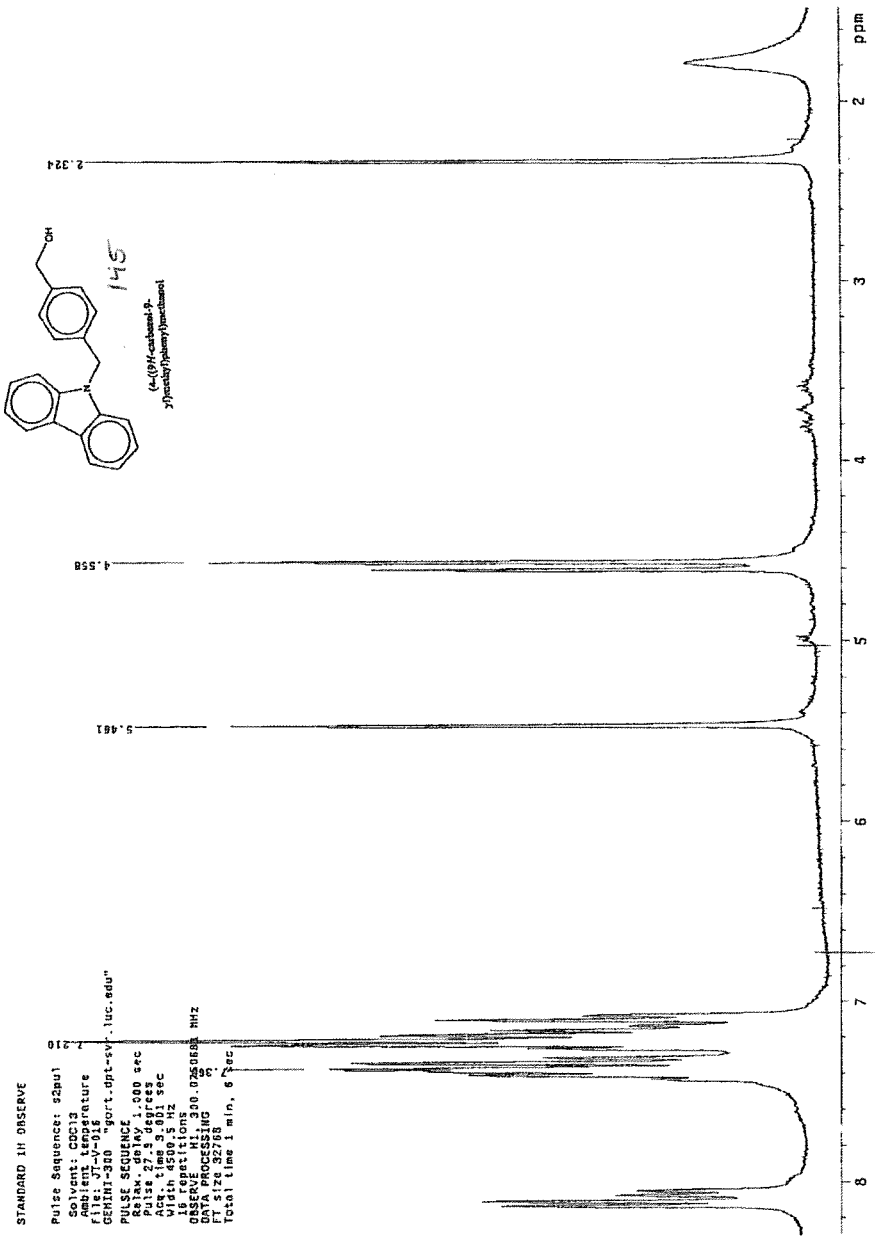
Pulse Sequence: s2pul
 Solvent: CDCl3
 Ambient temperature
File: JT-V-015
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
 Relax. delay 1.000 sec
 Pulse 27.9 degrees
 Acq. time 3.001 sec
 Width 4500.5 Hz
 16 repetitions
OBSERVE H1, 300.0750681 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec

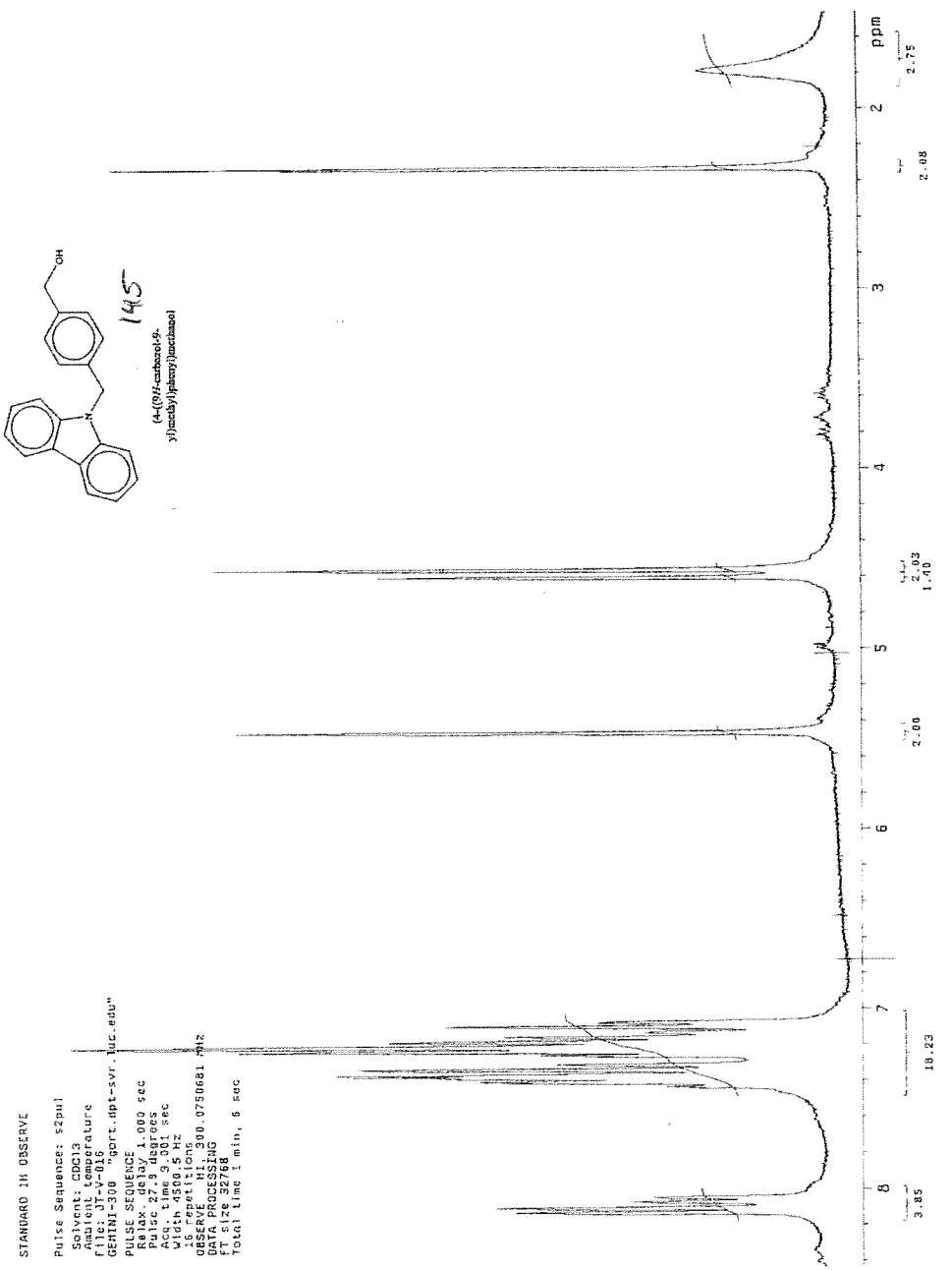


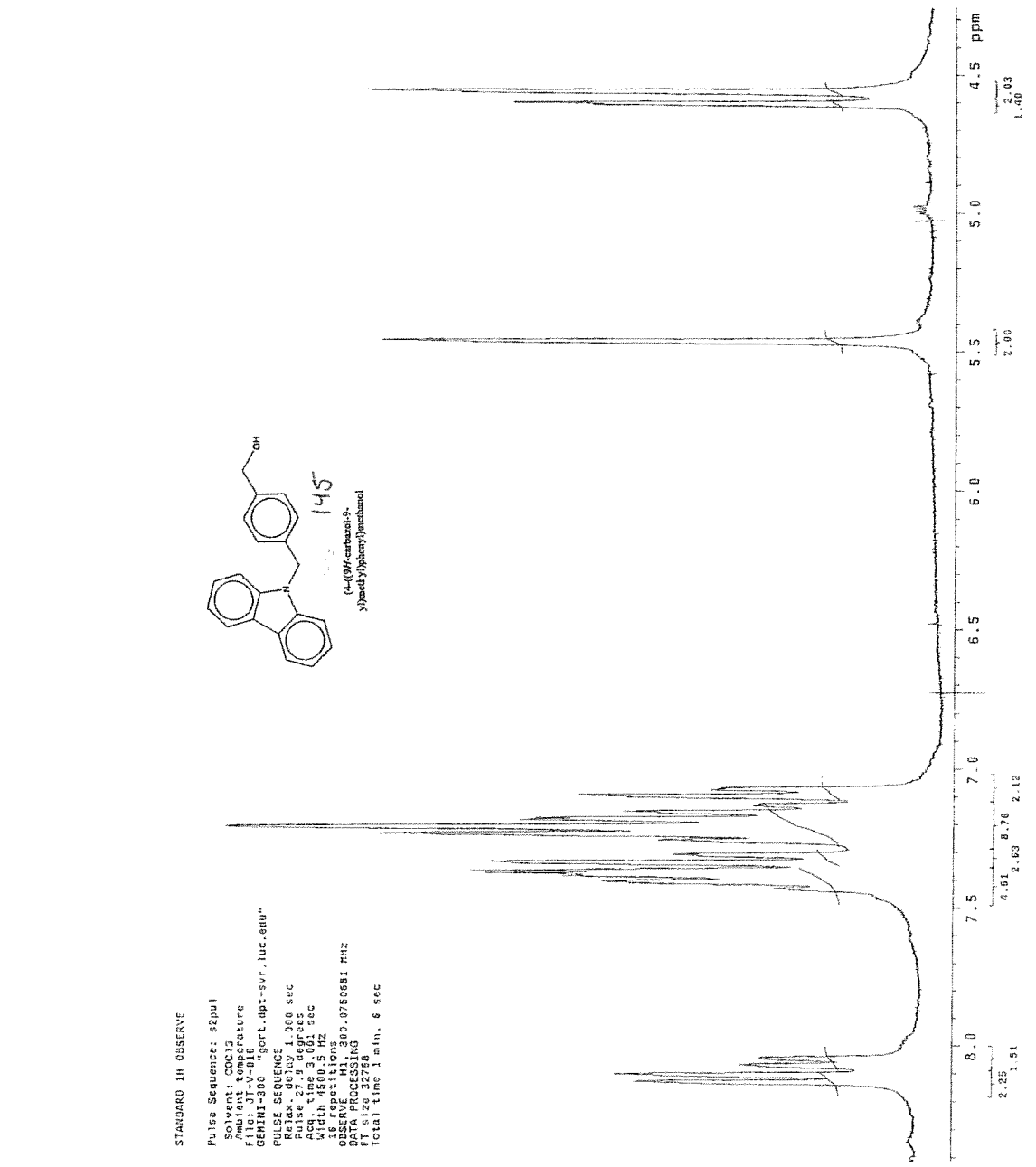
145

(4-((9*H*-carbazol-9-yl)methyl)phenyl)methanol



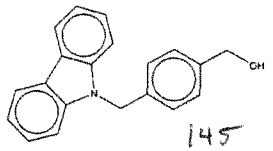

STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: rmc15
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750688 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
2.210
4.558
5.461
2.324
OH
ppm
1
2
3
4
5
6
7
8


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: JT-V-016
GEMINI-300 "gort.dpt-svr.tuc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4615.5 Hz
16 repetitions
OBSERVE H1, 300.0750681 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
OH
N
145
(4-((9H-carbazol-9-yl)methyl)phenyl)methanol
ppm
8
7
6
5
4
3
2
3.85
18.23
2.00
2.03
1.40
2.08
2.75


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
Flavio IT-WORKS
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
RE Pulse Delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750681 MHz
DATA PROCESSING
FT 32768
Total time 1 min, 6 sec
145
(4-((9H-carbazol-9-yl)methyl)phenyl)methanol
OH
N
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
ppm
2.25
1.51
4.61
8.76
2.63
2.12
2.00
2.03
1.40

STANDARD 1H OBSERVE

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: JT-V-016
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750681 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec

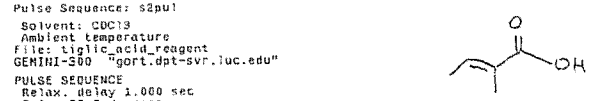


145

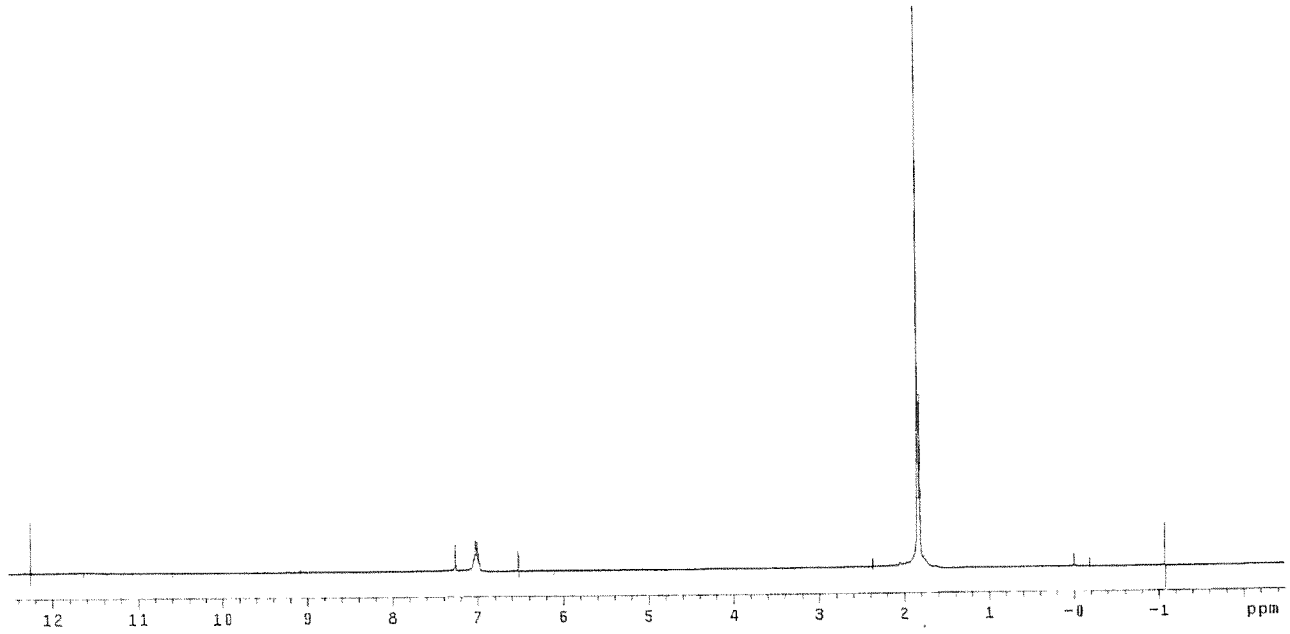
(4-((9*H*-carbazol-9-yl)methyl)phenyl)methanol

STANDARD 1H OBSERVE tiglic acid (REAGENT)

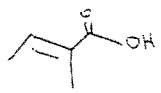
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: tiglic_acid_reagent
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750546 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec



154



tiglic acid (REAGENT)



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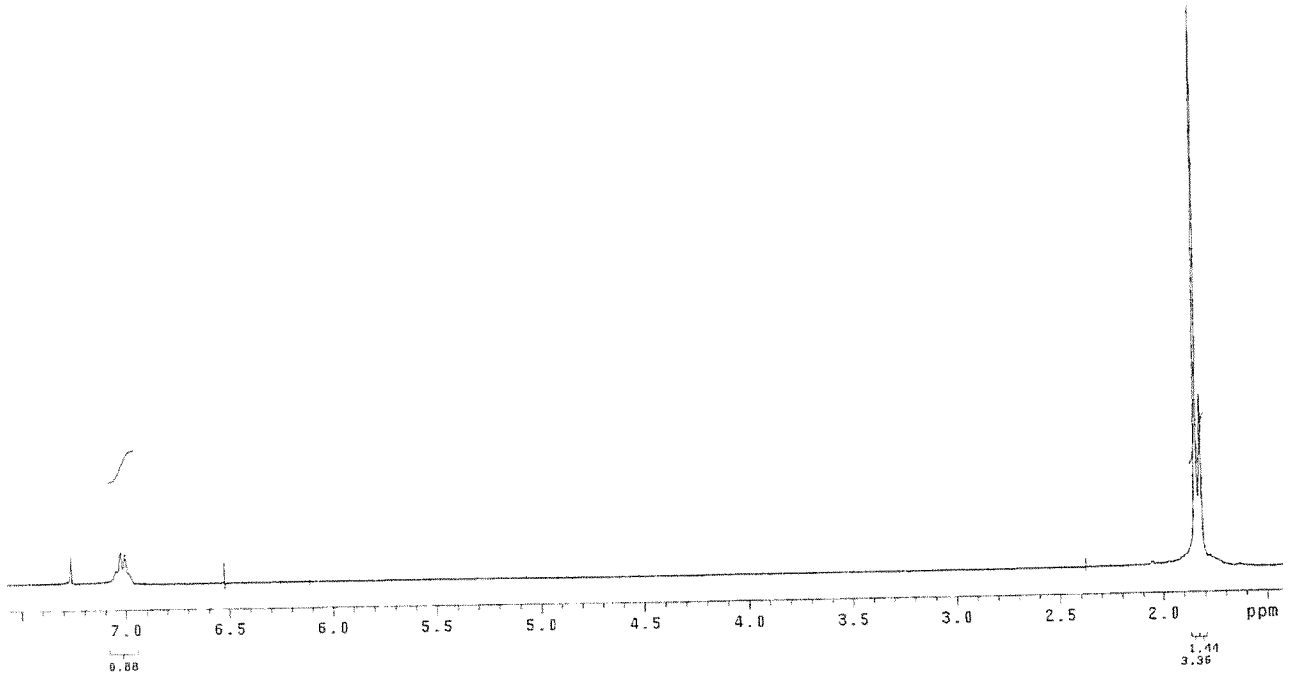
STANDARD 1H OBSERVE

Pulse Sequence: s2pul

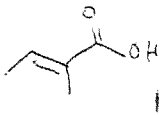
Solvent: CDCl3
Ambient temperature
File: tiglic_acid_reagent
GEMINI-300 "gort.dpt-svr.luc.edu"

PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.5 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750546 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec



Tiglic acid

(REAGENT)



154

STANDARD IN OBSERVE

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Solvent: CDCl3
Ambient temperature
File: tiglic_acid_reagent
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PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.5 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750546 MHz
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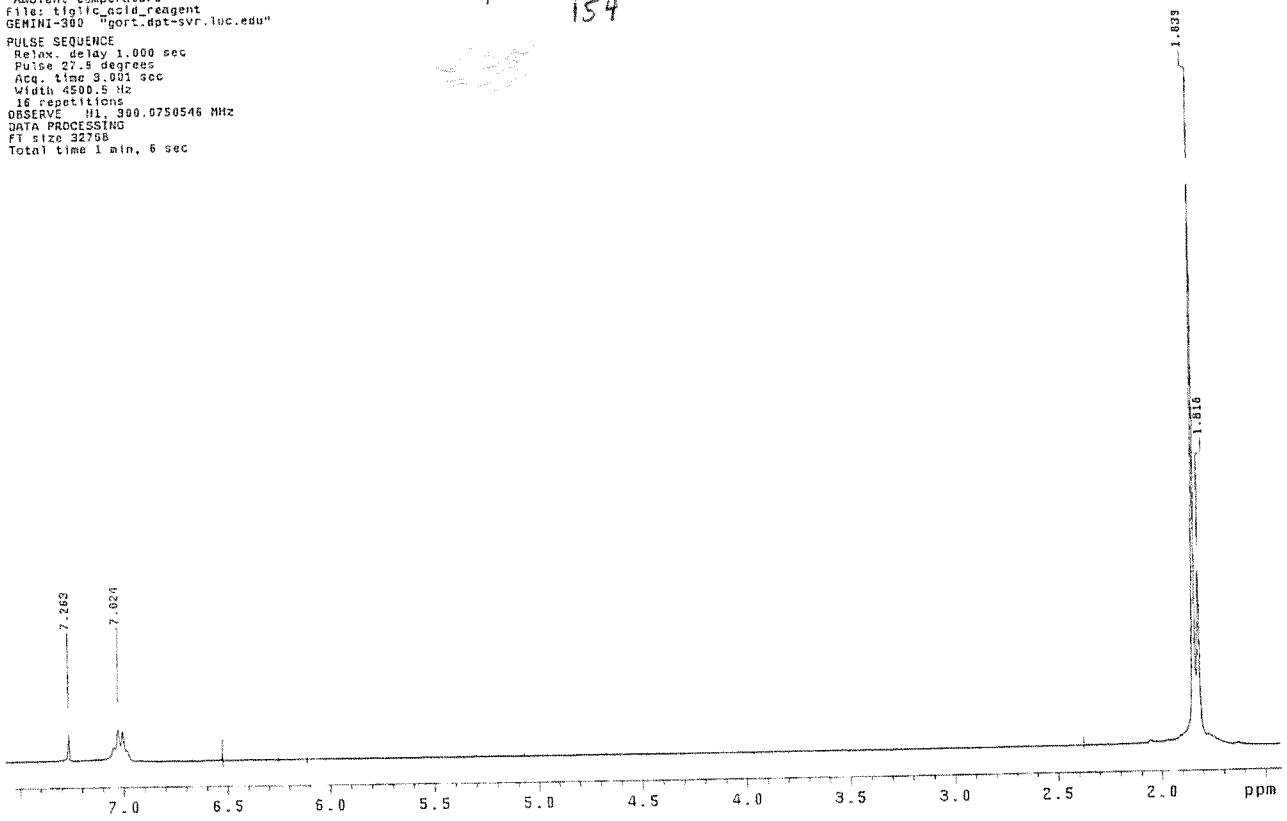
FT size 32768
Total time 1 min, 6 sec



ethyl ortho formate (REAGENT)

STANDARD 1H OBSERVE

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Solvent: CDCl3
Ambient temperature
File: triethyl_ortho_formate
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PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
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DATA PROCESSING
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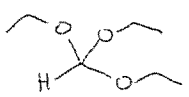
Total time 1 min, 6 sec



164

ethyl ortho formate (REAGENT)

STANDARD 1H OBSERVE

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Pulse 27.9 degrees
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Width 4500.5 Hz
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DATA PROCESSING
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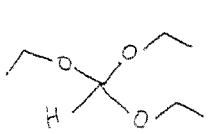
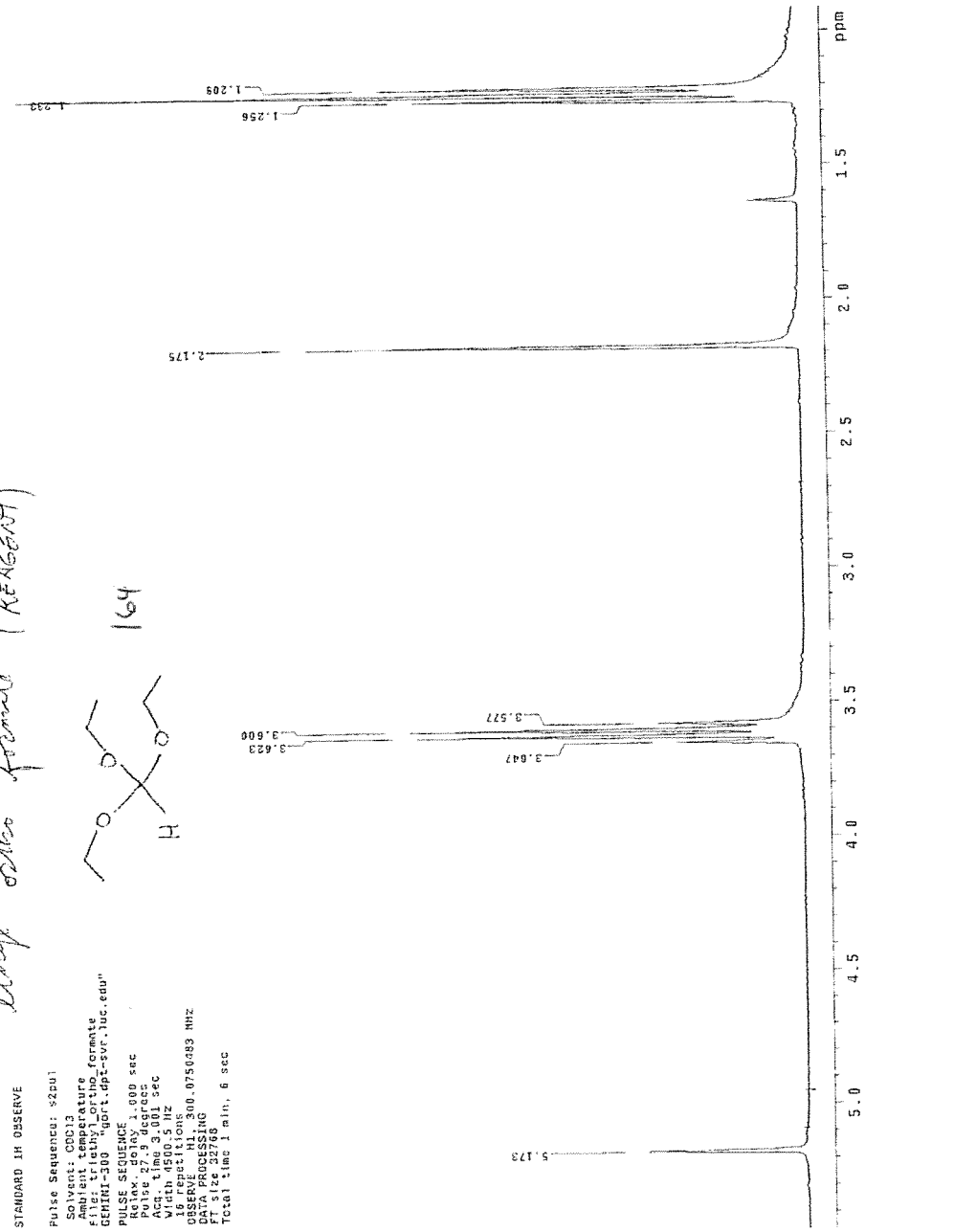
Total time 1 min, 6 sec



164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Ambient temperature
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Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
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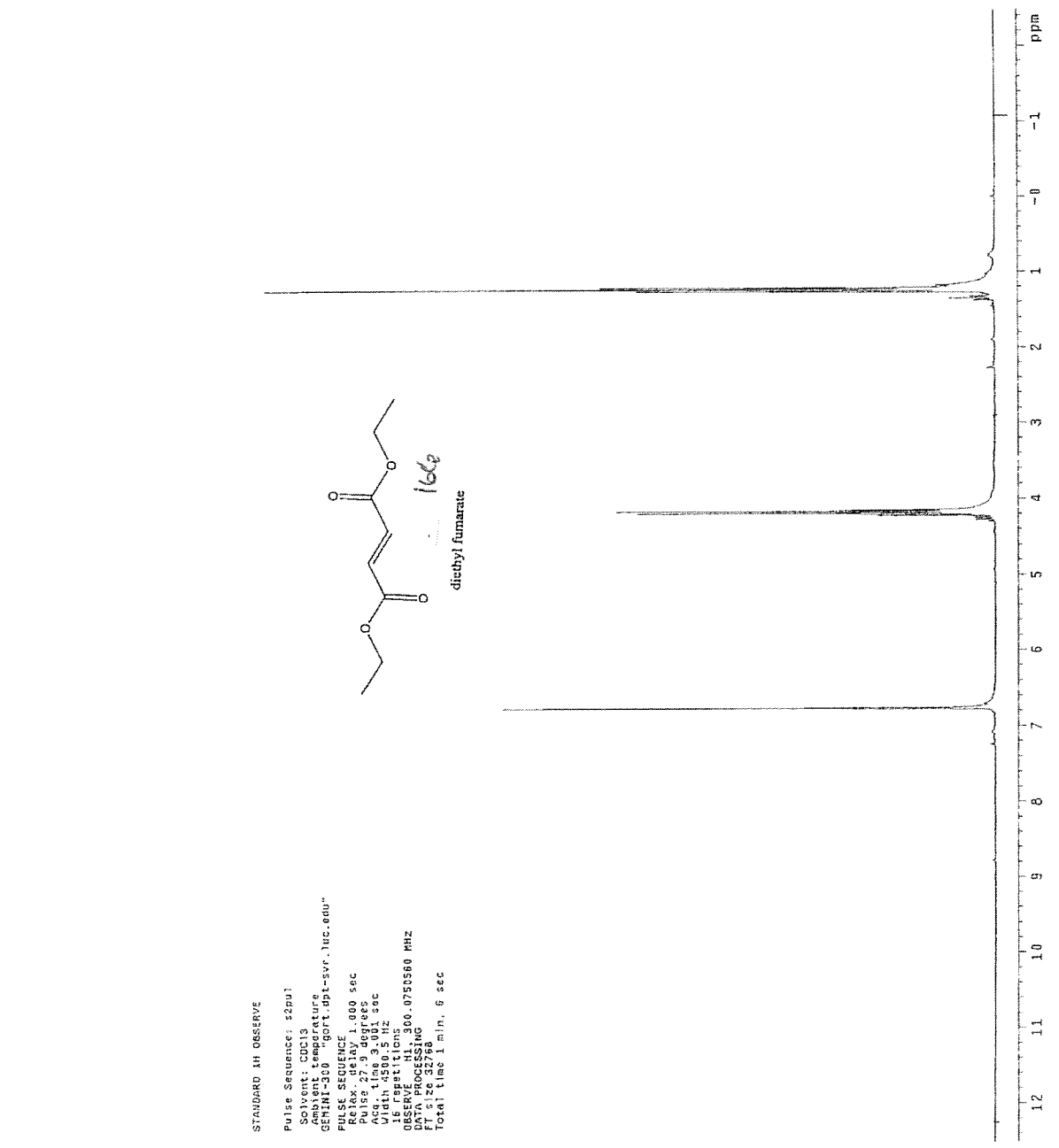
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
ethyl ortho formate (REAGENT)
164
1.233
1.209
1.256
2.175
3.623
3.600
3.577
3.647
5.173
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm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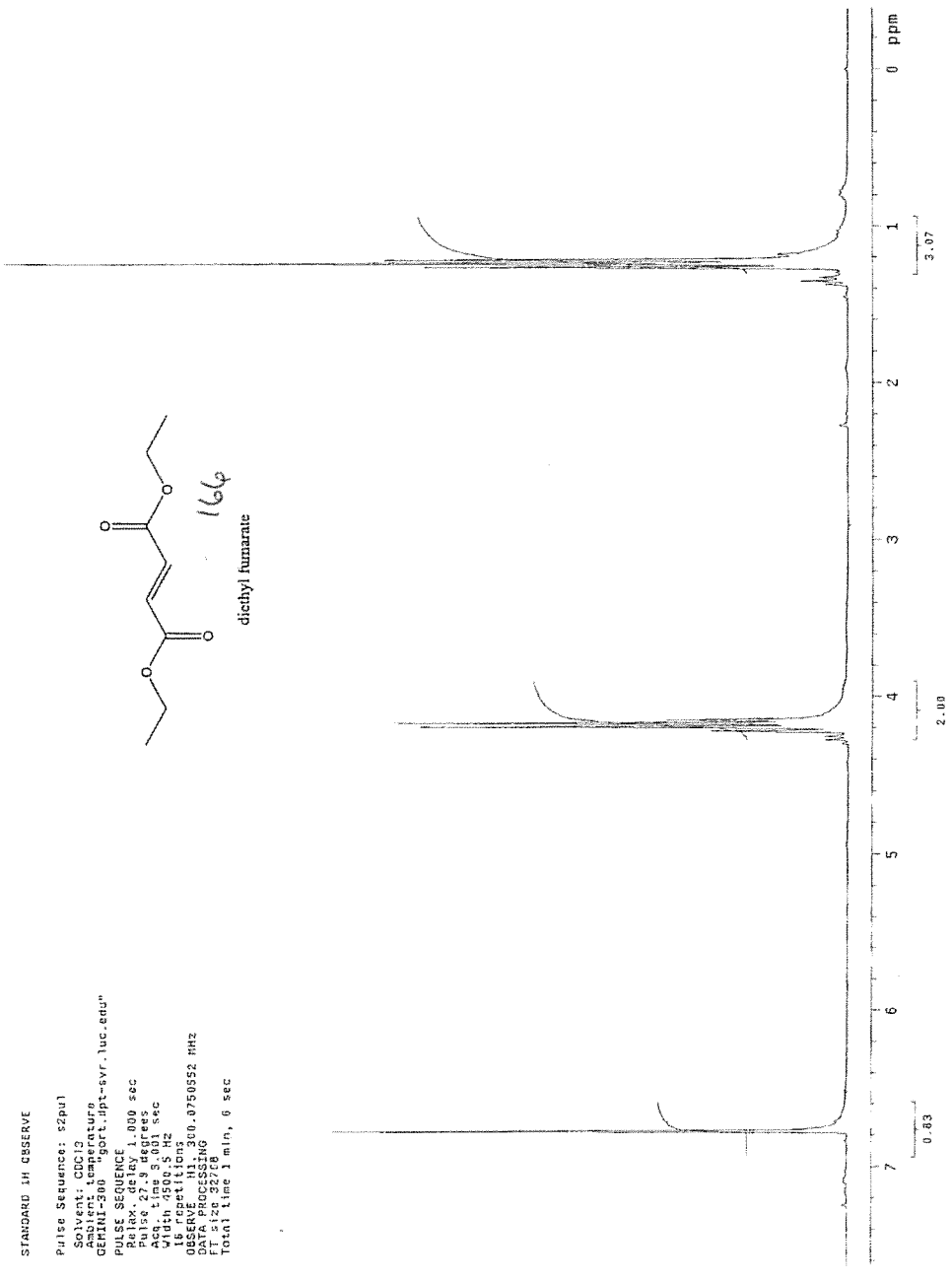
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750560 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
diethyl fumarate
ppm


STANDARD 1H OBSERVE
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Ambient temperature
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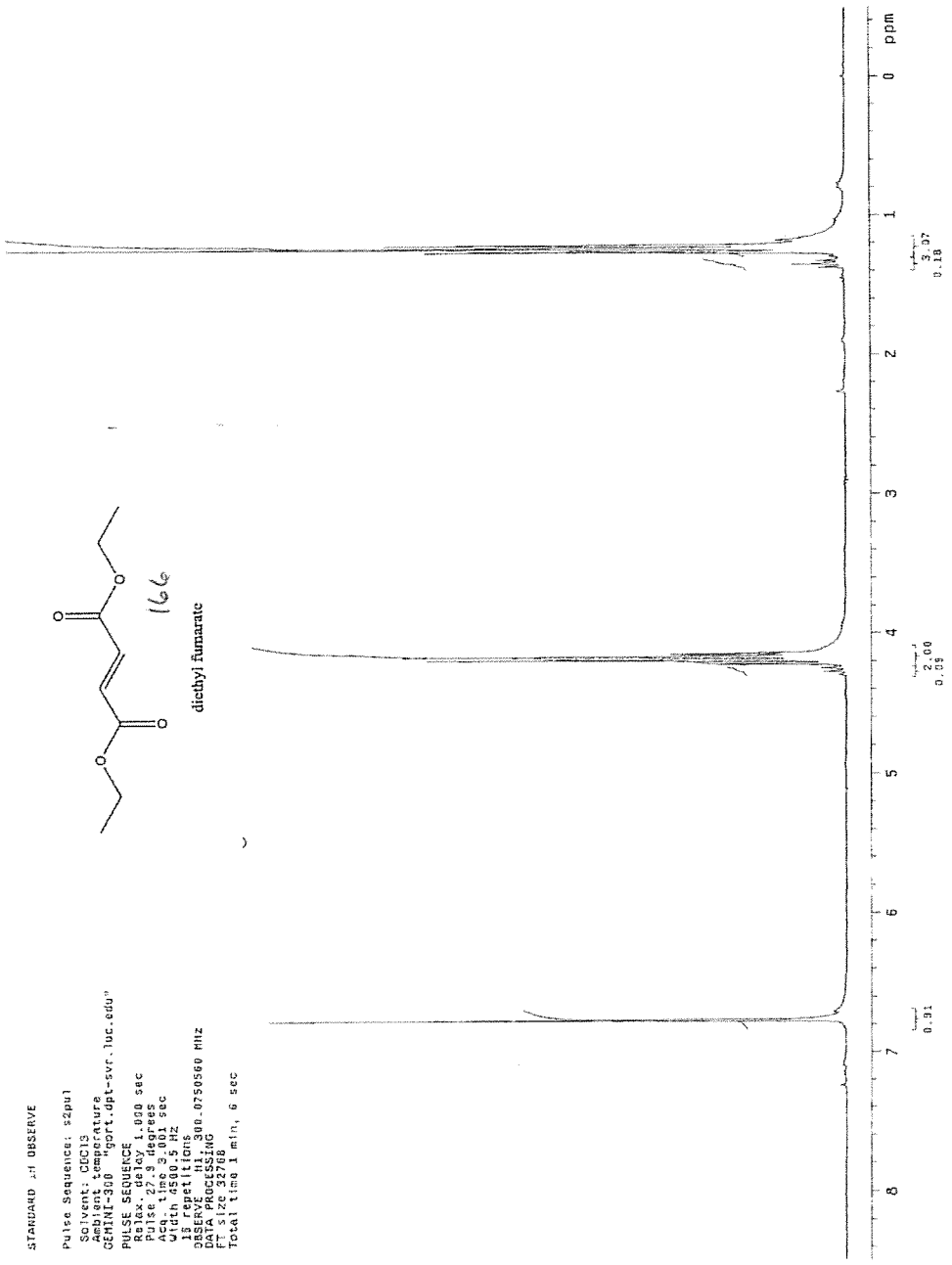
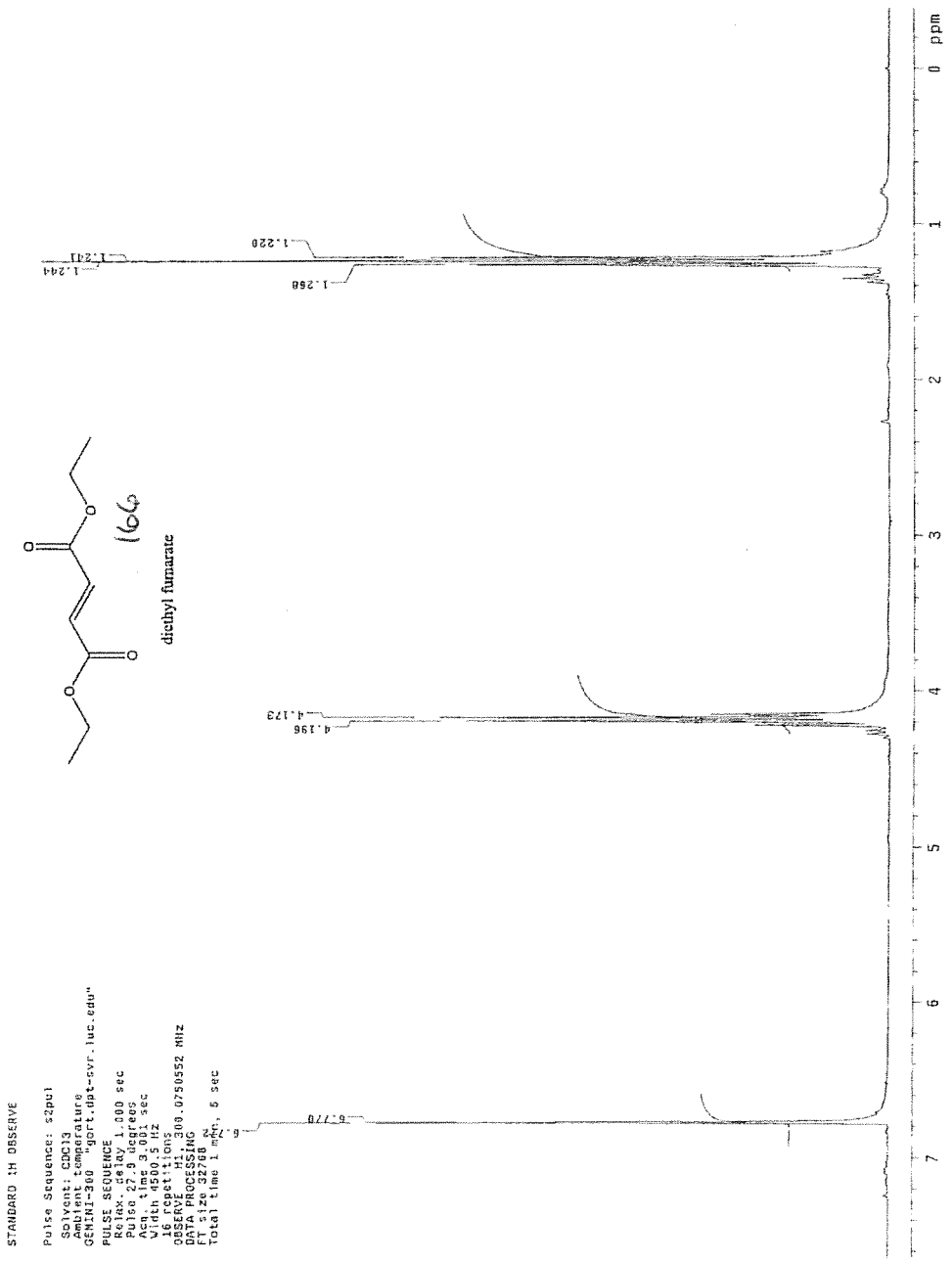
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 1500.5 Hz
16 repetitions
OBSERVE H1, 300.0750552 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
166
diethyl fumarate
ppm
0.83
2.00
3.07


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "port.dpt-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750560 MHz
DATA PROCESSING
FT 32768 points
Total time 1 min, 6 sec
O
O
O
O
O
O
diethyl fumarate
166
8
7
6
5
4
3
2
1
0 ppm
0.91
2.00
0.09
3.97
0.18


STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
GEMINI-300 "gort.dat-svr.luc.edu"
PULSE SEQUENCE
Relax. delay 1.000 sec
Pulse 27.9 degrees
Acq. time 3.001 sec
Width 4500.5 Hz
16 repetitions
OBSERVE H1, 300.0750552 MHz
DATA PROCESSING
FT size 32768
Total time 1 min, 6 sec
6.770
4.196
4.173
1.268
1.244
1.241
1.228
0 ppm
1
2
3
4
5
6
7
diethyl fumarate
164

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VITA

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Currently, Jeffrey is a tenured Associate Professor of Chemistry at College of DuPage in Glen Ellyn, Illinois. He resides in Naperville, Illinois.